





Digitized by the Internet Archive  
in 2008 with funding from  
Microsoft Corporation









P.  
Med.  
P.

# PHYSIOLOGICAL RESEARCHES

EDITED BY

BURTON EDWARD LIVINGSTON, Manager  
*The Johns Hopkins University*

DANIEL TREMBLY MACDOUGAL  
*The Carnegie Institution of Washington*

HERBERT MAULE RICHARDS  
*Columbia University*

VOLUME II

JULY, 1916—MAY, 1923

WITH SIXTY FIGURES

PHYSIOLOGICAL RESEARCHES  
*Laboratory of Plant Physiology  
of the  
Johns Hopkins University  
Baltimore, Maryland  
U. S. A.*

189708  
9.6.24

12M  
9

LIBRARY OF THE  
BUREAU OF THE  
NAVY  
WASHINGTON  
D. C.

2000  
12-11-11

COMPOSED AND PRINTED AT THE  
WAVERLY PRESS  
BY THE WILLIAMS & WILKINS COMPANY  
BALTIMORE, MD., U. S. A.

## Table of Contents of Volume II

	PAGE
The cryoscopic constants of expressed vegetable saps, as related to local environmental conditions in the Arizona deserts. <i>J. Arthur Harris and Johns V. Lawrence, with the coöperation of Ross Aiken Gortner</i>	1
Further studies on foliar transpiring power in plants. <i>A. L. Bakke and B. E. Livingston</i>	51
An analysis of the causes of variations in the transpiring power of cacti. <i>Edith B. Shreve</i>	73
A preliminary study of climatic conditions in Maryland, as related to plant growth..... <i>Forman T. McLean</i>	129
The influence of certain environmental conditions, especially water supply upon form and structure in <i>Ranunculus</i> ..... <i>Donald Folsom</i>	209
The quantitative determination of photosynthetic activity in plants. <i>Frances Louise Long</i>	277
A comparative study of the two races of <i>Rhizopus nigricans</i> ..... <i>Grace A. Dunn</i>	301
A physiological study of the climatic conditions of Maryland, as measured by plant growth..... <i>F. Merrill Hildebrandt</i>	341
The relation of acidity to carbon-dioxide adsorption by certain gels and plant tissues..... <i>Cornelia Lee Carey</i>	407



# THE CRYOSCOPIC CONSTANTS OF EXPRESSED VEGETABLE SAPS AS RELATED TO LOCAL ENVIRONMENTAL CONDITIONS IN THE ARIZONA DESERTS

J. ARTHUR HARRIS AND JOHN V. LAWRENCE

WITH THE COÖPERATION OF  
ROSS AIKEN GORTNER

## ABSTRACT<sup>1</sup>

The cell sap is both the product and the environment of the activities of the protoplast. Its chemical and physical properties should, therefore, be factors of fundamental significance in plant physiology. The validity of this proposition is attested by the extensive literature of turgor, osmotic pressure and permeability, which has resulted from the great activity of physiologists in the laboratory during the past half-century.

Notwithstanding the fact that certain of the properties of the fluids of plant tissues, particularly the cryoscopic and the electrolytic, may be determined with reasonable ease and a relatively high degree of accuracy, it does not seem to have occurred to the laboratory physiologists on the one hand or to the phytogeographers on the other, that a characteristic apparently so fundamental as the osmotic concentration of the tissue fluids might well be taken into account in any well organized ecological survey. To be sure, there are the pioneer studies of Drabble and Drabble on English habitats and of Fitting on North African deserts, and a number of minor contributions of data which repay comparative study. The suggestiveness of these observations tends, however, to emphasize the fact that even those ecologists who have attempted to measure in quantitative terms the physical factors of the natural environment, have been quite too content to record the characteristics of the organisms which are associated with these conditions in purely descriptive terms involving macroscopic and microscopic structures that are merely skeletal.

Those who suggest that the physico-chemical properties of the tissue fluids should be considered in phytogeographical work must of course show that the species of plants constituting different plant societies, formations, associations, or vegetations, —whatever the plant geographer or ecologist may care to call them,—are clearly differentiated with respect of these properties.

Such comparisons as can be made between the plants of the North African deserts, studied by plasmolytic methods (Fitting), and the plants of meso-

<sup>1</sup>The manuscript of this paper was received Mar. 1, 1916. This abstract was prepared, with corrections from these types and was issued as *Physiological Researches Preliminary Abstracts*, vol. 2, no. 1, July, 1916.

phytic and other regions, investigated by plasmolytic, cryoscopic and vapor tension methods, have indicated that the osmotic concentration of the sap of desert plants is, on the average, higher than that of forms growing in mesophytic regions. These conclusions have been confirmed by a general comparison of the Arizona data here presented, with determinations made in the neighborhood of Cold Spring Harbor, New York. Several as yet unpublished studies indicate marked differences in the osmotic concentration of the leaf sap of the species of strand, mangrove swamp, everglade and rain-forest vegetation.

The purpose of the study reported in this paper has been to determine whether, within a fairly closely circumscribed region showing clearly marked but not too extreme edaphic differences, the osmotic concentration of the tissue fluids of the plants characteristic of the several habitats shows measurable differentiation.

For such an investigation the region within working distance of the Desert Laboratory, at Tucson, Arizona, has proved an ideal locus, because of the diversity of conditions prevailing within a narrowly circumscribed area all of which may quite properly be called desert. The results of these Tucson studies demonstrate, first of all, that the species belonging to the several vegetation forms differ significantly in the osmotic concentration of their expressed sap, as measured by the cryoscopic method which we have employed. Thus, for the region as a whole the average cryoscopic determinations are:

	<i>Atmospheres</i>
Trees and shrubs.....	28.10
Dwarf and half-shrubs.....	21.45
Perennial herbs.....	16.35
Winter annuals.....	14.73

When the plants investigated are classified according to their habitats it is found that those of the arroyo or sandy wash show the lowest osmotic values. These values range from as low as 37 to nearly 100 per cent. of those obtained for plants of the same vegetation form but growing in other habitats. The plants of Pima canyon, of the foothills of the Santa Catalina mountains, exhibit lower osmotic values than those of any other habitat, with the exception of the arroyo. The plants of the rocky slopes are characterized by osmotic values of their expressed sap higher than that of the canyon and the arroyos but lower than those of the bajada, or mesa-like slopes, and of the salt spots. The plants of the bajada give sap of higher osmotic concentration than do those of any other habitat except the salt spots. Finally, the plants of the alkali or salt spots have the highest concentrations of all, as might have been expected. Here the osmotic values are from 1.12 to 2.71 times as high as in the case of plants from the other habitats studied.

The explanation and interpretation of these differences involve a problem of great complexity, which demands further investigation.

## INTRODUCTORY REMARKS

The physico-chemical properties of the cell sap of an organism are in large degree dependent upon the environmental conditions to which it is exposed. The proof of this statement is to be found in a mass of published data due almost exclusively to laboratory experimentation. It does not seem to have occurred to plant physiologists on the one hand or to ecologists on the other that the results of attempts to modify the properties of the cell sap by controlling and varying external conditions should also be tested out in the field.

Yet it seems quite reasonable to assume that by the amassing of large series of determinations in environmentally clearly differentiated habitats, one should be able to ascertain something of the rôle of varying external conditions in determining the properties of the cell sap of plants as they develop under natural conditions.

The importance to be attached to the results of laboratory research must depend largely upon the results of such field studies. If variations in the properties of the cell sap comparable to those which may be induced in the laboratory are not to be found in nature as a result of varied environmental conditions, such laboratory experimentation must, we believe, lose much of its significance in plant physiology. If such variations do occur in nature, the problems presented by them pass out of the exclusive boundaries of plant physiology and come within the province of, and become of fundamental importance to, the ecologist, phytogeographer and evolutionist.

If one suggests that the study of the concentration (in molecules and ions) and other physico-chemical properties of vegetable saps should be taken up as one of the regular details of ecological work, he must show (*a*) that the properties of the sap of plant species differs from region to region, or (*b*) that in a given region variations in the properties of the sap are associated with local ecological conditions, or that *a* and *b* are both true.

If, as the result of such an attempt to carry these studies into the field, one can demonstrate but a slight interdependence between external conditions and internal state, it would be unreasonable to expect students of phytogeography to devote much attention to these problems. If, on the other hand, the properties of the fluids contained in the tissues differ widely from habitat to habitat, just as the more superficial features and the microscopic structure of the plant tissues have long ago been shown to do, it will seem quite proper to regard the physico-chemical properties of the tissue fluids of the plant as characteristics as fundamental as the more obvious macroscopic and microscopic features, and to consider a knowledge of them as essential to a thoroughgoing ecological study as is a recounting of morphological details. To us they seem far more significant. Differences in gross morphology, in so far as they concern the ecologist, are generally interpreted in reference to

cellular structure and arrangement. Differences in microscopic anatomy deal for the most part with the skeleton of the cell. The cell sap, however, is intimately related to its every fundamental activity. The cell membrane separates a sap of given physical and chemical properties from a soil solution or an intercellular atmosphere presenting quite different conditions. In this cell sap the cytoplasm must carry out all its synthetic and metabolic activities and the nucleus must grow and divide. It is the fact that all the activities of the protoplasm, both somatic and germinal, must take place in the environment of the cell solution, which has particularly interested those of us who are primarily concerned with the problems of organic evolution.

This paper is the first of a series in which we hope, by presenting the results of quantitative studies of plant species growing in highly diversified but natural conditions as contrasted with experimentally controlled conditions, to lay the foundations for a comprehensive knowledge of the relationship between the chief ecological and phytographical factors and the physico-chemical properties of the sap of the species of plants constituting the vegetation.<sup>2</sup> The limitation just indicated implies no criticism of the method of controlled experimentation. For many purposes such methods are indispensable. It is a restriction drawn for temporary convenience merely, but with the conviction that both lines of work are supplementary and equally indispensable to the most valuable results. The fact that most of our information has been derived from laboratory experiments lends emphasis *for the moment* to the importance of field studies.

In an earlier paper (Harris, Lawrence and Gortner [1915]) we compared the Arizona series here presented in detail with a set of determinations from Long Island. The results sufficiently demonstrate, we believe, that the vegetations of the two regions are profoundly differentiated not merely in the taxonomic and morphological sense, but in the physico-chemical properties of their juices as well.

Our purpose in the present paper is to consider the problem of the association of differences in the freezing point of vegetable saps with physiographic and other edaphic features of a particular environment. If we succeed in showing that within the range of conditions presented by a fairly closely circumscribed region, there are easily discernible differences in the

---

<sup>2</sup> In further explanation of the presentation of these data in their present form we may say that we hope ultimately to investigate a rather wide range of typical plant environments. As a matter of fact the constants here presented are only about one-tenth of those which we have in our notes. The carrying out of these studies as planned will require some years for completion. When these wider series of constants are analyzed we hope to discuss in a comparative way all the available data, by whatever method secured. At the same time we hope to show the relationship of the available data to the results of laboratory experimentation on the one hand and to those of comparative ecology on the other. It would be premature to attempt such discussion until all the evidence *which* seems to be forthcoming are ready for consideration.

For the moment, two lines are of paramount importance: (1) to determine whether such results as those presented in these pages are really of significance in ecological and phytogeographic work, and (2) to place on record the actual constants for the use of botanists working in any field.

physico-chemical states of the fluids in which the physiological activities of the organism must be carried on, we shall have shown that a determination of the properties of the sap should form a part of any intensive ecological research.

### HISTORICAL REVIEW

A detailed history of the work on the properties of vegetable saps falls quite outside the scope of the present paper. As we have already indicated our knowledge of the subject is due chiefly to experimental work. Among plant physiologists, ecologists and geographers wide interest was therefore aroused by the publication of Fitting's studies [1911] on the osmotic pressure of the cell sap of desert plants. These followed closely the lines laid down by the work of two English investigators (Drabble and Drabble, [1907]) carried out four years earlier. The results obtained by Fitting and by Drabble and Drabble indicate that in a given region the osmotic pressure of the cell sap is in some degree correlated with local differences in available soil moisture. The work of Fitting has received wider recognition largely because of two facts. First, he worked under the advantageous conditions presented by a desert environment. Second, he reported concentrations of cell sap far higher than those familiar to most botanists. This phase of Fitting's work has even been called in question by various physiologists who were apparently unfamiliar with the splendid cryoscopic studies carried out several years ago at Cagliari by Cavara [1905].

The investigations of Cavara, of Drabble and Drabble and of Fitting suggest that in any region variations in the osmotic concentration of the cell sap are associated with edaphic differences. Marie and Gatin [1912] have called attention to the difference between the freezing points of the saps of three species studied by them in lowland and alpine habitats. Halket [1913] has furnished certain data for salt marsh plants. Fitting, by a comparison of his series of determinations from the neighborhood of Biskra with such measurements as were available for other regions, concluded that the osmotic pressure of desert plants is far higher than that of those flourishing in mesophytic regions. In many regards, however, the series of determinations made thus far leave much to be desired. Drabble and Drabble and Fitting used plasmolytic methods; Marie and Gatin determined the freezing point lowering; Halket employed a vapor tension method due to Barger.

The series of cryoscopic determinations made by Cavara [1905], Dixon and Atkins [1914] and Ohlweiler [1912] all suffer from the disadvantage—as viewed in their relation to the present inquiry—of being based largely upon plants growing outside their natural environments. All of the earlier determinations (other than plasmolytic) were made on sap extracted by pressure alone from untreated tissue. As Dixon and Atkins (Dixon [1914]) and we ourselves (Gortner, Lawrence and Harris [1916]) have shown, such methods

may lead to very erroneous results. These comments are in no sense in disparagement of these pioneer studies, but merely in explanation of our decision to carry out such investigations upon a far more extensive scale, in a wider series of habitats, by one and the same method, and if possible with greater precision of results. To demonstrate that the plants of different floras, plant associations, societies or whatever the nomenclatorial specialist may choose to call them are differentiated in more than structural features, is, it seems to us, only the first step. If such differences exist they should be quantitatively measured, and finally correlated with the already familiar taxonomic or environmental differentiations.

## METHODS

In carrying out this study it has been necessary to consider: (a) selection of habitats, (b) choice of plant species and organs to be examined, (c) collection of samples, (d) technique of determination of final constants, and (e) the accuracy of the coefficients.

(a) *Selection of habitats.* As a locus for the kind of study proposed here the Tucson region offers three distinct advantages: (1) It presents such diversity in edaphic conditions that if differences in the properties of saps are correlated with them, the relationship should be clearly demonstrable by the comparison of series of determinations made in the various ecological habitats. At the same time the relationships are not so *a priori* probable as, *e.g.*, those which one might expect to find in comparing the constants obtained from plants growing on the margins of streams with those occurring in the salt marshes of our mesophytic Atlantic seaboard.

(2) All of these diverse habitats together constitute one of the typical desert regions. Thus the determinations made to solve the problem discussed in this paper furnish the first of a number of series of data required for a comparison of the larger phytogeographic regions. One of these comparisons has already been drawn in a preliminary way (Harris, Lawrence and Gortner, [1915]). Others which are made possible by our unpublished data indicate their great interest.

(3) There is the highly important feature of an equipped base of operations. Without the assistance and facilities given us by the Director of the Department of Botanical Research this piece of work could not have been carried out. Without the ever generous suggestions of Dr. Cannon and Dr. Shreve who placed the results of their long experience in the southwest freely at our disposal, it would have fallen short in more points than it has.

In our desire to meet the requirement of securing a series of determinations as representative as possible of the desert as a whole, we did not limit collections to the most typical portions of the local habitats to be discussed below, but took them everywhere and classified later. Since one natural hab-

it passes imperceptibly into another, there is, in many instances, some question as to the disposition which should be made of an individual determination. We feel sure that in the great majority of cases any other field botanist would agree with the classification made. The transitions between the local habitats as well as the typical portions of each have furnished their quota of determinations. The differences between the local habitats as given in the averages in our tables are probably not so large as they might have been had transition zones been disregarded and had collections from selected localities only been taken.

(b) *Choice of plant species and organs to be examined.* In the Tucson region there are two seasons of vegetative activity. Our work was done entirely during the period of winter annuals and of herbaceous and ligneous perennials. In another study we hope to deal with the species characteristic of the summer season.

The succulents and certain of the hard-leaved species are more or less active throughout the year. It so happened that during the period that we were working at Tucson the winter annuals and herbaceous perennials were in unusually good condition. We felt it necessary, therefore, to devote our attention especially to these and to the ligneous species coming into leaf at the same time, leaving the cacti, which are available at every season of the year, for study when conditions are less favorable for work on the more ephemeral species.

The root hairs and the cells of the leaf (or the physiological homologues of the leaf) are those which are most directly exposed to the influence of varying environmental conditions. To investigate with any degree of accuracy the concentration of the sap of the root cells would have presented a problem of the greatest difficulty. Our work has, therefore, been practically confined to the tissues of the leaf. In making this limitation we have necessarily had to consider questions of practicability. In the few cases in which the leaves were wanting, or were so minute that the collection of a sample was impossible, in which the gathering of the leaves would have been so slow that greater sources of error would have been introduced, or in which the stems were negligible in volume or demonstrably of the same character, the whole shoot was taken. In such cases the flowers, unless very minute, were removed to avoid the possible influence of nectar. Whenever feasible we gathered our materials from plants in flower in order to have them in as nearly comparable stages of development as possible.

As may be seen from statements below, it has sometimes been impossible to determine the *age* of the leaves employed. This is a difficulty unavoidable in a study carried out during only a portion of the year, in a semitropical region with the distribution of rainfall found in the Southern Arizona deserts.

(c) *Collection of samples.* As already stated, our experience, like that of

Dixon and Atkins, has proved that very erroneous constants may result from determinations made upon sap extracted by pressure merely. For this reason most of our material was frozen as soon as gathered and thawed again only when the extraction was to be performed. The tissues to be investigated were gathered as rapidly and with as many precautions as possible against contamination, and placed in thick-walled test tubes of about 100 cc. capacity. These were kept tightly closed, except when introducing additional material, to preclude loss of water by evaporation. In many of the collections the temperature of the leaves was at once reduced by a portable apparatus for the evaporation of ether in a Dewar bulb surrounding the specimen tube. This procedure added very greatly to the labor of collections, in many cases excessive at best, and in some instances it could not be employed. As soon as possible after collection the tubes were plunged in a mixture of ice and salt where they were subjected to a temperature of about  $-17^{\circ}$ , and kept frozen until thawed for the extraction of the sap (Gortner and Harris [1914]).

In the case of a few of the species, which, because of the exceeding hardness or dryness of their leaves, had to be collected in large quantities, the leaves were simply securely packed in paraffined paper bags and brought from the field to the laboratory for freezing. The leaves of one or two of the forms which are known to remain practically unchanged for a long time (*Yucca* and *Agave*), were brought in without protection. In all instances, however, the time intervening between the collection of the tissue and its freezing was reduced to the lowest practicable minimum.

(d) *Determination of freezing-point lowering.* The data of this paper pertain exclusively to the depression of the freezing point of extracted sap. Because of considerations, which need not be detailed here, the cryoscopic method was adopted as a standard for use throughout the series of studies to which the present paper belongs.

The juice was extracted from the thawed tissue by means of a small, heavily tinned beef-juice press or, when the small press was not sufficiently powerful, by means of a large screw press capable of exerting a pressure of about 80–100 atmospheres. In a few cases it was necessary to reduce the tissue by grinding or by crushing on an anvil, as well as by freezing, before sap could be extracted. The juice was then centrifuged to clear in so far as possible. The freezing point lowering was determined by means of an ordinary Beckmann thermometer employed in connection with a simple apparatus for the evaporation of ether in a Dewar bulb.

The thermometer was read in most cases to thousandths of degrees. Correction for under-cooling was made as suggested elsewhere (Harris and Gortner [1914]) and the results expressed in freezing-point lowering in degrees C. ( $\Delta$ ), and osmotic concentration (pressures) in atmospheres ( $P$ ), according to calculated tables (Harris and Gortner [1914] and Harris [1915]).

(e) *Accuracy of coefficients.* The measurements recorded in this paper are not constants in the sense in which the physicist or chemist would use the term. The freezing point of the sap is not a constant for a species or organ, but it is a value which is the resultant of many variables. The concentration of the sap is to some extent influenced by the age of the organ, by the time of day at which the sample is collected, by prevalent methodological conditions, and so on. In laboratory experimentation these factors may, within limits, be controlled, but even such limited control is impracticable in field studies. Indeed, the time is probably far distant when it will be possible to apply corrective factors to determinations made in the field. We have therefore omitted from this paper many details which were noted in making the collections.

It will be quite evident, too, that the technique is such that the constants have not the exactness of those made under more favorable conditions. To the man who has never worked outside the laboratory, the materials may seem unsuitable and the technique rough and ready. To no one are the possibilities or the sources of errors more apparent than to us who did the work. We must, therefore, insist that the degree of precision to be sought must be conditioned by the nature of the materials and the kind of problem with which one is dealing. Even in the laboratory one does not water a plant potted in soil with conductivity water in order to secure a greater precision in measuring transpiration. No one can ever repeat our work under precisely the same conditions. Except by accident no one will ever get *exactly* the same thermometer readings as we have. However, anyone repeating the work will, we feel confident, secure results approximating our own within reasonably close limits. In the long run our general conclusions will, we believe, be substantiated.

## SALIENT CHARACTERISTICS OF THE REGION SELECTED

The salient physiographic and vegetational features of the deserts of the southwest have been so well described by a number of writers, particularly since the establishment of the Desert Laboratory that any detailed account in this place would be superfluous. Those unacquainted with the literature may find the most comprehensive and best terse description in a guide leaflet prepared by Shreve [1914] for the use of the International Phytogeographic Excursion in America, 1913. More detailed and splendidly illustrated descriptions are to be found in the reconnaissance work of Coville and MacDougal [1903] or the more recent account by MacDougal [1908]. In a paper intended for the general, rather than the botanically trained, reader one of the present writers has emphasized what seemed to him the most fundamental biological characteristics of the region (Harris [1916]).

The geology of the general region of Tucson has been reviewed by Blake

(in MacDougal [1908], pp. 45-68), while that of Tumamoc hill has been treated by Tolman in greater detail (in Spalding [1909], pp. 67-82). It has, however, seemed quite premature to attempt any discussion of the possible relation of the sap properties to the geological nature of the substratum.

We have found Livingston's early paper on soil conditions [1906] and Cannon's work on root habits [1911] particularly useful. For a discussion of the plant societies of the Desert Laboratory domain, from which a considerable proportion of our collections was made, the work of Spalding [1909] may be consulted. The *Flora of New Mexico* by Wootton and Standley [1915], just issued from the United States National Herbarium, has served us in many ways.

In view of the splendid series of illustrations published in the papers cited above we have considered plates of the vegetation of the different habitats superfluous.

## PRESENTATION AND ANALYSIS OF DATA

We now proceed with our attempt to determine, by an appeal to actual measurements, whether, in the Tucson deserts studied by us, differences in the osmotic concentration of the expressed sap may be demonstrated to be associated with local differences in environmental conditions.

Early in our work it became apparent that the freezing point of the sap of a species is in some degree correlated with its growth form. To this subject we hope to return in a special paper when our determinations for the species of other regions are more fully worked up. For the present it is sufficient to avoid the dangers of heterogeneity of data by classifying the constants with which we have to deal according to growth form. How these differ for the *spring flora* of a *particular region* the reader may ascertain by a comparison of the entries in the tables near the end of this paper.

Growth forms are not sharply defined. To avoid any suspicion of personal bias, the classification of such materials as are here dealt with should be *a priori*, not *a posteriori*, with regard to the environmental factors. It is unquestionably desirable to have some standard of classification already fixed before work is undertaken. To eliminate in so far as possible all personal equation we have followed Thornber (in Spalding [1909], pp. 103-112) as closely as possible in our classification of vegetation forms. The method of grouping of classes will be seen in the headings below.

Fortunately, we have been able to take Spalding's [1909] detailed study of the habitats and plant associations of Tumamoc hill as a basis of local environmental classification. His study covered only the Tumamoc hill portion of the region from which our collections were made. In order to include all of the types of habitats from which we were able to secure materials we have had to expand his classification in certain directions. In other cases we have

found it necessary to group closely similar units of his classification in order to obtain series of determinations sufficiently large to justify the calculation of averages and the drawing of comparisons. The modifications of Spalding's classification will be made clear below. No criticism whatever of his grouping is implied. Change for our purposes has been a matter of necessity, not of choice. Spalding's [1909] grouping is as follows:

1. The River and Irrigating Ditches: Association of Hygrophytes.
2. The River Banks: Association of Cottonwoods and Willows.
3. The Flood Plain: Mesquite Forest Association.
4. Salt Spots: Association of Salt Bushes.
5. The Wash: Palo Verde—Cat Claw Association.
6. The Mesa-like Slope (Sub-divided into Associations).
7. The Hill (Sub-divided into Associations).
8. Superficial Soil Layers: Association of Annuals.
9. Parasitic and Symbiotic Plants.

Of these nine associations, the first two were untouched by our field studies. Such samples as we derived from the flood plain were so far back toward the mesa-like slopes that they can be equally well assigned to it. The reasons for neglecting these habitats are perhaps obvious. The plants of the irrigating ditches and of the banks of the Santa Cruz are apparently as hygrophytic or mesophytic as those of the Eastern United States. During the spring season the conditions with regard to soil moisture on the flood plain are not exceedingly severe. Thus while a detailed investigation of the characteristics of these forms for comparison with those of the mesa-like slope and the other localities would be desirable, it did not seem so profitable during the few weeks we had in the field, as the work on the more characteristic desert plants.

The fourth, fifth and sixth habitats of Spalding's classification we have maintained. Probably he or any other ecologist would have classified our collection from salt spots and from the arroyo just as we have done. With regard to the collections from the mesa-like slopes there is opportunity for decided differences of opinion. The mesa-like slope grades, on the one hand, into the flood plain, and, on the other, into the rocky detrital slopes.

Because of the fact that our collections extended over a considerable territory we have been unable to retain Spalding's hill association, which after all, is, according to his own subdivisions, quite heterogeneous. Nor have we felt it feasible to recognize a flora of the superficial soil layers, the association of annuals. Instead, we have felt it wise to consider the annuals in the same habitats as the perennials. We have, however, in a later section of this paper drawn comparisons between the properties of the sap of annuals and perennials.

It has seemed expedient to recognize, in addition to the three cases in which we have followed Spalding exactly, two associations of plants, that of

the cliffs and rocky slopes and that of the foot-hills canyon. The first of these includes little more than the hill association of Spalding, but lumps together annuals and perennials. The second has been made necessary by work outside of the range of his study.

Thus the local environments, which we shall consider in their possible relations to the physico-chemical properties of the saps, are five in number. We arrange them in an order slightly different from that adopted by Spalding—an order which seems to us to represent more truly a genetic sequence, as determined by the processes of base leveling.

- A. The foot-hills canyons.
- B. Cliffs or ledges and steeper rocky slopes.
- C. The bajada or mesa-like slopes.
- D. The arroyo or wash.
- E. Salt spots.

The salient physical and vegetational features of these habitats, in so far as they can be outlined from our own observations and from published data, will be discussed under their respective headings before the constants determined from collections made in them are taken up. The constants will then be presented in tabular form and discussed.

#### A. THE FOOT-HILLS CANYONS

The Santa Catalina mountains rise from the desert floor, with an elevation of between two and three thousand feet and with conditions of extreme dryness, and reach to an elevation of over eight thousand feet, with rain and snow sufficient for a luxurious mesophytic vegetation. In the foot-hills canyons of these mountains there is an interesting mixture of these extremes of environment and vegetation.

In Pima canyon, where our collections were made, the contrasts which one would naturally expect in such a transition region are perhaps heightened by exposure. The canyon opens towards the southwest. Thus in the spring the temperature is higher and vegetative activity and flowering are earlier than in other localities of the same elevation. The range of surface presented by the transitions from the rocky walls to the small tracts of almost flat floor and the transient stream that flows during the spring months is doubtless accompanied by a corresponding range in soil moisture conditions. As the vernal season advances the stream disappears as a surface feature, but it is quite possible that during most of the year the ligneous plants which grow among the rocks along its banks are able to draw upon an ample water supply while their aerial portions are exposed to an atmosphere of high evaporating power and intense insolation.

A further discussion of the highly complex conditions presented by such a locality as Pima canyon is not necessary here. These various features have received thorough attention at the hands of Shreve [1915].

## TREES AND SHRUBS

*Dodonaea arizonica* Thornber ined. $\Delta = 2.47, P = 29.6$ 

Not far from the transient stream. Leaves apparently developed early in the current season, but concerning this point we could not be certain.

*Hyptis emoryi* Torr.Apr. 3,  $\Delta = 0.96, P = 11.6$ 

Leaves of plants in flower among rocks on floor of canyon, not far from temporary stream.

*Lycium*, species undeterminedMar. 12,  $\Delta = 1.72, P = 20.7$ 

Leaves apparently of the spring growth, but most of the leaf-bearing axes were well lignified.

*Populus*, species undetermined(1) Mar. 13,  $\Delta = 1.87, P = 22.5$ (2) Apr. 3,  $\Delta = 1.86, P = 22.4$ 

Our collections were made from the lowermost clump of trees which had attained a splendid development with their roots in the transient stream. Wootton and Standley [1915] say of the species of *Populus* occurring in New Mexico "They are all rather short-lived trees and grow in stations where the soil is at least moderately wet, preferring the broad river valleys . . . or locations besides mountain streams or springs." The first collection was made when the leaves were still young and waxy; the second determination was made from the more mature leaves. Professor Thornber still has the species under consideration.

*Simmondsia californica* Nutt.Apr. 3,  $\Delta = 3.39, P = 40.6$ 

(1) Mature leaves of plants which had nearly passed flowering. The plants were at this time sending out young shoots bearing new leaves which had attained about the normal dimensions but were still tender, much thinner than those which had persisted over the winter and were green instead of grey in color. These gave :  $\Delta = 2.26, P = 27.1$

*Vitis arizonica* Engelm.Apr. 3,  $\Delta = 0.80, P = 9.6$ 

Dr. Shreve tells us that this grape is confined to the bottoms of the canyons in the same type of localities as those occupied by the cottonwoods. Wootton and Standley give its New Mexican habitats as canyons and thickets in the upper Sonoran and Transition zones. The leaves used, apparently not quite mature, were from a large, flowering specimen.

## DWARF SHRUBS, HALF-SHRUBS AND WOODY TWINERS

*Chrysoma laricifolia* (A. Gray) Greene

$$\Delta = 2.07, P = 24.9$$

Dryest places near the mouth of the canyon. The leaves collected were almost entirely of the spring growth. They were very resinous and sap was extracted with some difficulty. The values given must be taken as only approximate.

*Cissus macrocarpa* Rose

$$\Delta = 0.71, P = 8.6$$

The very fleshy leaves of this species, which occurs among rocks near the transient stream, give a gelatinous juice.

*Gaertneria ambrosioides* (Cav.) Kuntze

$$(1) \text{ Mar. 14, } \Delta = 1.43, P = 17.2$$

$$(2) \text{ Apr. 3, } \Delta = 1.88, P = 22.6$$

This species occurs in considerable abundance in the mouth of the canyon. Collections were made from plants in flower and young fruit. Two types of leaves were collected—those from the short flowering shoots and those from the long vegetative shoots, the most of which would probably bear flowering branches the following season. The latter are much larger than the former. The age of the leaves presented a question of considerable difficulty. The majority were doubtless developed during the spring, but there were evidences that some dated from an autumn period of growth. This two-fold dimorphism rendered the securing of really typical samples rather difficult, and may account for the somewhat divergent results of the two determinations.

*Gaertneria cordifolia* (A. Gray) Kuntze

$$\text{Mar. 13, } \Delta = 1.37, P = 16.4$$

Among rocks, frequently associated with the preceding species near the mouth of the canyon. Leaves, some of them apparently of the previous season's growth, chiefly from flowering branches.

*Isopappus coronopifolia* (A. Gray) Greene

$$\text{Mar. 13, } \Delta = 2.23, P = 26.8$$

Young shoots, the spring growth, were very resinous and squeezed with difficulty. The determination is open to some question because of a delay in extraction of sap, due to the breaking of our large press.

## PERENNIAL HERBS

*Euphorbia*, species undetermined

$$\text{Mar. 13, } \Delta = 1.19, P = 14.4$$

Forming small, dense tufts from a rather large woody root, in the dryer parts of the floor of the canyon. Because of the small size of the leaves the entire flowering shoot was taken.

*Nicotiana trigonophylla* Dunal

Mar. 12,  $\Delta = 1.35$ ,  $P = 16.3$

Radical and cauline leaves of flowering plants, near stream.

*Pentstemon wrightii* Hook

(1) Mar. 13,  $\Delta = 1.12$ ,  $P = 13.5$

(2) Apr. 3,  $\Delta = 1.09$ ,  $P = 13.2$

Radical and cauline leaves of plants growing near the stream.

*Villanova dissecta* (A. Gray) Rydb.

$\Delta = 1.00$ ,  $P = 12.0$

A single specimen with a few flowers, among rocks near the water-course. Some of the leaves had apparently been retained from a fall growth, others expanded in the spring of the current year.

#### WINTER ANNUALS

*Galium aspernum* A. Gray

(1) Mar. 13,  $\Delta = 0.80$ ,  $P = 9.6$

(2) Apr. 3,  $\Delta = 0.80$ ,  $P = 9.6$

Flowering plants growing in the moistest habitats, among rocks near the stream in shaded places. Because of the smallness of the leaves the whole shoot was used. The results from the two collections are identical, to the number of decimal places recorded in this paper.

*Gilia floccosa* A. Gray

Mar. 13,  $\Delta = 1.29$ ,  $P = 15.5$

Associated with the three following species in the dryer portions of the floor of the canyon. Because of the smallness of the leaves the entire shoot was taken.

*Linanthus bigelovii* (A. Gray) Greene

Mar. 13,  $\Delta = 1.48$ ,  $P = 17.8$

Associated with *Gilia floccosa* and the two following species in the dryer, more gravelly parts of the floor of the canyon. Because of the minute size of the leaves the whole shoot of flowering plants was taken.

*Platystemon californicus* Benth.

Mar. 13,  $\Delta = 1.12$ ,  $P = 13.4$

Associated with the two preceding and with the following species in the more level, drier portions of the floor of the canyon. To a considerable extent this species occurs in the shade of shrubs and there are evidences of its very ephemeral nature. Probably it completes its development very rapidly during the spring periods of more abundant moisture. Leaves of flowering plants.

*Sphaerostigma chamaenerioides* (A. Gray) SmallMar. 13,  $\Delta = 1.15$ ,  $P = 12.3$ 

Occurs with the three preceding winter annuals in the more level and drier parts of the canyon floor. The plants were very small as compared with those collected from the arroyos, and it was with considerable difficulty that a sample of leaves was obtained.

*Vicia exigua* Nutt.Mar. 13,  $\Delta = 1.02$ ,  $P = 12.3$ 

Not far from the stream, in protected places. Here the plants, in flower, were considerably larger than the occasional examples found in the more protected places among the rocks on the upper slopes of Tumamoc hill. The stem, as well as the leaves, was taken.

## COMPARISONS OF THE FOOT-HILLS CANYONS WITH OTHER HABITATS

Only six species secured in the canyon could be obtained elsewhere for analysis, during the time at our disposal.

The shrub *Hyptis emoryi* shows a slightly higher osmotic pressure on the rocky cliffs of Tumamoc hill than it does in the canyon, where it was collected not far from the transient stream.

*Gaertneria ambrosioides* does not normally occur on Tumamoc hill, but one individual, probably introduced in horse feed, was found. This gave a concentration intermediate between the two Pima canyon determinations.

The perennial herb, *Nicotiana trigonophylla* is rather widely distributed. It occurs chiefly where the supply of water is fairly large. The three collections, one from the canyon where it grew close to the transient stream, one from a rocky slope near Agua Verde creek where it probably had considerable seepage water from higher levels, and one from the mesa not far from the Rillito river where it probably received some water from the higher bajada, all give pressures lying within a range of one and one-half atmospheres.

The splendid perennial herb, *Pentstemon wrightii*, found among rocks on the banks of one of the gullies opening into Agua Verde creek, gave a slightly greater depression than in Pima canyon.

The winter annual *Sphaerostigma* gave slightly greater freezing-point lowering in Pima canyon than in the Tumamoc or Agua Verde creek arroyos.

The winter annual *Gilia floccosa*, taken from an apparently very dry slope in the canyon, shows an insignificantly higher concentration than the same species from the large arroyo on Tumamoc, and a distinctly higher pressure than the constant determined from the collection made on Agua Verde creek.

From these few cases no final conclusion can be drawn concerning the differences between the same species in the canyon and in other habitats. The conditions within the canyon are far too complex for such comparisons to be of validity except when carried out on a very extensive scale.

## B. CLIFFS OR LEDGES AND STEEPER ROCKY SLOPES

The cliffs or ledges here described are for the most part not precipitous walls of rock with here and there a cluster of plants occupying a seam of soil, but are rather the bold' outcroppings of rock on the more or less precipitous mountain sides piled with large blocks of stone—the most recent product of the disintegration of the solid substances of the mountains. There is no sharp line of demarcation between the rocky ledges and the steeper rocky slopes. Genetically, the former are the progenitors of the latter, just as the rocky slopes in their turn give origin, through weathering and erosion, to the more finely ground substratum of the mesa.

The soil, in such localities on Tumamoc hill, is a fine or "heavy" adobe clay, malpais, derived from the volcanic rock, with the admixture of about an equal amount of small particles of volcanic rock and "caliche," a calcareous hardpan. This soil with its admixture of finer rock particles is distributed among the larger rock blocks and the outcropping ridges of the rock foundation in such a manner that fully half of the first half meter or so is solid rock.

The adobe clay puddles readily, and hence is decidedly impervious to water. This condition, in connection with the large surface exposure of undisintegrated rock, results in the loss of a very high percentage of the precipitation water in superficial "run-off." Notwithstanding the fact that the scanty rainfall is thus largely lost by surface drainage, the soil in the crevices between the larger rock masses of these ledges presents probably the most mesophytic conditions (or at least the most persistent mesophytic conditions) found away from the river banks and transient streams of the foot-hill canyons. Protected as the soil and the annual and smaller perennial plants are from the direct rays of the sun, evaporation and transpiration must be much reduced as compared with habitats of less irregular surface.

The water-holding capacity of the clays of Tumamoc hill is also very high, as demonstrated by Livingston [1906] (p. 17). Here as on the mesa-like slopes is found a layer of caliche at the depth of a meter or less which probably is another factor contributing to the maintenance of soil moisture among the masses of larger broken rocks in that it fairly effectually seals many of the soil pockets formed by adjoining rocks. The extent and depth of these pockets varies greatly. In the deeper pockets considerable water is probably retained even during the driest seasons.

That there are also great differences in the water content of the deeper-lying soil layers of Tumamoc is evidenced by the behavior of *Fouquieria* as noted independently by Livingston [1906] (p. 12), Harris, and perhaps many others. The behavior of this plant in leaf production and leaf fall and in flowering is a most sensitive indicator of soil moisture, as the observations of many and the experiments of Lloyd [1905] show. The plants are exceedingly variable in the time of leaf production, as soil moisture is being in-

creased by precipitation, in time of leaf fall, as induced by soil desiccation, and in the time of flower production. Experimentally, the foliation of the plant is quickly induced by slight irrigation. Variations in leaf production and loss and in anthesis as they are observed in the open are therefore most logically explained by variation in the water content of the substratum. It is the opinion of those who have studied the question most thoroughly, Spalding, Cannon and Livingston, that although the surface layers become exceedingly dry during the driest season of the year and are thus quite unsuited to the growth of shallow-rooted annuals or to the germination of seeds, the lower layers contain nevertheless sufficient water for the life and even growth of the deeper rooted perennials.

Livingston [1906] (pp. 10-11) found that at the height of the dry summer season the sun-baked surface of the clay soil on the shoulder of Tumamoc hill in the vicinity of the Laboratory contained about 2 per cent. of water, by volume, but that at a depth of 10-35 cm. it contained from 7 to 15 per cent. Thus at the driest season of the year the soil does contain rather large amounts of water, and this at no great depth.

Cannon [1911] (pp. 16-17) summarizes the conditions of moisture supply on the hill thus: "The soil to a depth of 15 cm. probably does not retain sufficient moisture for absorption by plants whose roots do not reach deeper than this for a period much exceeding six weeks following storms. This defines the limit of life of most annuals, both those of winter and those of summer, and probably also the season of absorption of perennials with shallowly placed roots. Plants which have roots which reach to greater depth than 15 cm. can probably obtain some moisture at all seasons. In order to survive, seedlings must send their roots below 15 cm. within six weeks following the close of a stormy period."

A factor which seems to us of considerable importance in the water supply of the plants growing upon the rocky slopes is drainage or seepage from higher levels. Underlaid as so much of the soil is with a relatively impervious hardpan, "caliche," there would necessarily be a considerable flow from the higher to the lower slopes. Livingston [1906] (pp. 11-13) has emphasized the importance of rock surfaces in leading the water of rock pockets into the soil.

Later Livingston (in Spalding [1909], p. 85) gave direct evidence of the reality of a sub-surface flow of water. After unusually heavy November and December rains which had probably as nearly saturated the soil of the hill as it ever becomes, the soil lying on the caliche layer was found to be saturated in places, and in excavation work the water was found to be actually flowing along the caliche and lava. That the caliche is generally impermeable to water is shown by the fact that the soil below the layers which were thus conducting off the water was practically air dry. Thus Livingston concludes that "in those rare instances where a rainy season furnishes sufficient water to nearly saturate the whole soil mass of the hill, a considerable portion of

the water originally absorbed is probably eventually lost by subterranean run-off."

The water thus lost to the soils of the more precipitous ledges must either be brought near enough to the surface to be available to plants growing on the gentler rocky slopes below, or be carried off and lost at a level deeper than is attained by most plants. We are convinced that the former is often the case. Our observations are to be sure only superficial, but they have been made in the field first hand and are both numerous and careful. It is this conviction, that many of the gentler but rocky slopes have a larger available supply of water than the less rocky portion of the bajada, that has led us to class the ledges and more rocky slopes together.

#### TREES AND SHRUBS

*Dasyllirion wheeleri* S. Wats.

$$\Delta = 2.92, P = 35.0$$

The specimen from which the leaves were collected grew among rocks on the edge of Agua Verde creek. It is rather difficult to decide whether it should be assigned to the rocky slopes or to the arroyo. Probably it was in a position to receive far more water than the majority of the plants of the species. The only method by which we succeeded in extracting sap from *Dasyllirion* was to reduce the leaves, which are exceedingly hard and tough, on an anvil before freezing and then to extract the juice. The determination is therefore open to some question, but we believe the concentration recorded is too low, rather than too high.

*Fouquieria splendens* Engelm.

$$(1) \text{ Mar. 29, } \Delta = 0.86, P = 10.4$$

$$(2) \text{ Apr. 2, } \Delta = 1.20, P = 14.4$$

It is a matter of keen regret that the individuals of this remarkable plant, which has received so much attention from desert physiologists, were not in condition for a thorough study of sap concentration while we were at Tucson. The first collection was of yellowish green leaves which were just beginning to develop rapidly. None of the plants had fully green leaves, and the most of them were certainly not more than half grown at that time. The second sample was taken when the leaves were considerably larger and darker green in color, though not fully mature. Thus the best determination we could secure is probably distinctly too low. Making due allowance for this fact, however, it is very interesting to note that this form which, although one of the most successful desert plants, is physiologically and in leaf structure a mesophyte,<sup>3</sup> has also a sap concentration more typical of mesophytic ligneous perennials.

<sup>3</sup> The activities of *F. splendens* are so closely related to the soil conditions that it may be taken practically as an indicator of substratum moisture. Leaves are quickly expanded after adequate precipitation or supplemental irrigation whatever the season of the year, if temperature be favorable, and are lost again when the supply is no longer adequate.

*Hyptis emoryi* Torr.Mar. 29,  $\Delta = 1.13$ ,  $P = 13.6$ 

On Tumamoc hill; the plants had been badly killed back, probably by the cold of the preceding winters. Leaves of flowering shoots.

*Lippia wrightii* A. Gray $\Delta = 1.43$ ,  $P = 17.1$ 

Our two collections of this species were made from the rocky ledges on the north slope of Tumamoc hill and from large plants in Tumamoc arroyo. The shrubs on Tumamoc hill from which our sample was taken had been badly killed back, probably by recent severe winters. The leaves were taken almost exclusively from recently developed lateral shoots from the old stems.

The osmotic pressure of the sap is not far different from that of ligneous mesophytic perennials. In this connection Spalding's and Blumer's notes on distribution are of considerable interest. According to Spalding [1909] it occurs almost exclusively on northern exposure slopes on Tumamoc but is variable in local distribution elsewhere. Summarizing Blumer's observations he says: ". . . thus, at elevations of 3000 feet and under, it grows only in protected places of north aspect, but loses its aspect preference at altitudes approaching 5000 feet; while at those near 6000 feet it is definitely limited to southern exposure . . . . At the lower levels it requires protection from the severe desert conditions, and finds this on sheltered rocks of northern aspect. At the higher altitudes, apparently requiring protection from cold, it finds a congenial home on warm southern exposures, while at intermediate elevations in the neighborhood of 5000 feet, the extreme conditions of both higher and lower altitudes are so far modified that it grows on all exposures."

*Lycium berlandieri* DunalMar. 26,  $\Delta = 1.96$ ,  $P = 23.6$ 

From Tumamoc hill.

*Yucca macrocarpa* (Torr.) Engelm.Apr. 17,  $\Delta = 1.62$ ,  $P = 19.5$ 

From rocky east slopes of a small peak on Agua Verde creek. Here the plants probably receive a considerable supply of water through seepage from higher levels. They were just coming into flower—considerably in advance of individuals in other localities.

*Zizyphus lycioides* var. *canescens* A. GrayApr. 7,  $\Delta = 2.74$ ,  $P = 32.9$ 

During the time we were at Tucson this shrub had nowhere, as far as we observed, fully expanded its leaves. Two samples of young leaves from

shrubs on the rocky slopes and on the banks of the Agua Verde creek arroyo gave insufficient sap for individual determinations and were combined. Since the leaves were not fully mature the determinations are probably distinctly too low.

DWARF SHRUBS, HALF SHRUBS AND WOODY TWINERS

*Calliandra eriophylla* Benth.

Apr. 10,  $\Delta = 1.33$ ,  $P = 16.0$

In cracks and crevices of rocks on slope fully exposed to the sun, Agua Verde creek. Plants nearly through flowering, leaves apparently not fully developed.

*Coleosanthus coulteri* (A. Gray) Kuntze

Mar. 10,  $\Delta = 1.68$ ,  $P = 20.2$

In protected spots among rocks on the north slope of Tumamoc hill. Plants apparently badly killed back by the cold of the preceding winters. Leaves from shoots, usually terminated by flower buds, of the spring growth.

*Encelia farinosa* A. Gray

(1) Tumamoc hill, Mar. 2,  $\Delta = 2.10$ ,  $P = 25.2$

(2) Tumamoc hill, Mar. 16,  $\Delta = 2.27$ ,  $P = 27.3$

(3) Tumamoc hill, Mar. 17,  $\Delta = 1.91$ ,  $P = 22.9$

(4) Tumamoc hill, Mar. 17,  $\Delta = 2.50$ ,  $P = 30.0$

(5) Mouth of Pima canyon,  $\Delta = 2.05$ ,  $P = 24.6$

(6) Agua Verde creek,  $\Delta = 2.21$ ,  $P = 26.6$

*Encelia* is one of the most characteristic of the desert perennials, but is rather sharply limited in its distribution. Spalding [1909] (p. 18) noted the intimate association of *E. farinosa* and *Carnegiea gigantea* on southern slopes, and their practical absence on northern slopes of Tumamoc hill. In a quadrat study of the opposite slopes of a deep erosion gulch northwest of the Laboratory he found 112 plants in 300 square meters on the slope facing west of south whereas in the same area on the shady side only 3 plants were seen. From all the evidence available Spalding concluded that *Encelia* is a form which is limited in its distribution to localities presenting conditions of more intense heat but relatively abundant soil moisture. Thus it is restricted to the steeper rocky slopes, where there is some reserve of water in the rock pockets, extending very rarely to the gentler detrital slopes where the substratum is more homogeneous and better drained.

Cannon [1911] (p. 46-48) and MacDougal and Cannon [1910] (p. 13), who confirm Spalding's [1909] statements regarding local distribution, give certain additional information of interest concerning the species. The root system is a generalized one, with depth extension determined by the nature of the soil. The large leaves which are borne towards the ends of the branches are dropped when arid conditions set in. Leaves and flowers are formed whenever water and temperature relations are favorable.

The Tumamoc hill samples were taken from a rocky, eastern slope near the Laboratory. The first sample of March 17 was taken at about sunrise. The sample of March 2, and the second sample of March 17 were taken at the period of maximum daily temperature. The sample of March 16 was taken at about 5 p.m. Thus the concentration seems to be, as one might expect *a priori*, distinctly related to diurnal variations in external conditions.

*Gaertneria ambrosioides* (Cav.) Kuntze

$\Delta = 1.53, P = 18.4$

This species, which has already been discussed from Pima canyon, does not normally occur on Tumamoc hill. The present determination was made from a single specimen, probably grown from seed introduced in feed.

*Gaertneria deltoidea* (Torr.) Kuntze

Apr. 2,  $\Delta = 1.78, P = 21.3$

Leaves of fruiting plants from rocky slope southeast of the Laboratory, Tumamoc hill.

*Parthenium incanum* H. B. K.

Apr. 7,  $\Delta = 2.04, P = 24.5$

Agua Verde creek, whorls of young but probably mature leaves.

*Psilostrophe cooperi* (A. Gray) Greene

Mar. 7,  $\Delta = 1.05, P = 12.6$

Young leaves of shoots from old roots—only a few flower buds visible. Tumamoc hill.

*Trixis angustifolia* var. *latiuscula* DC.

(1) Mar. 20,  $\Delta = 1.69, P = 20.3$

(2) Mar. 30,  $\Delta = 1.35, P = 16.3$

The two determinations, both from mature leaves of flowering plants on Tumamoc hill, differ considerably. The lower value was obtained from plants growing among and protected by masses of broken rocks. Here the soil was to a considerable extent protected against evaporation, and possibly derived water by seepage from higher levels.

#### PERENNIAL HERBS

*Arabis eremophila* Greene

$\Delta = 1.22, P = 14.6$

Plants in nearly mature fruit, Agua Verde creek. The leaves taken were mostly mature ones of the spring's growth, but some recent ones which were not quite mature and some which had developed the preceding autumn were probably included.

*Artemisia ludoviciana* Nutt.

Apr. 7,  $\Delta = 1.11$ ,  $P = 13.4$

Agua Verde creek, often in crevices of rocks on slopes fully exposed to the sun. Plants not in flower, the age of the leaves could not be determined.

*Bahia absinthifolia* var. *dealbata* A. Gray

Mar. 27,  $\Delta = 1.17$ ,  $P = 14.1$

Northern exposure of Tumomac hill where the species is rather infrequent. Leaves of flowering plants. The difference between this determination and one from the Agua Verde creek bajada is so great that one is inclined to question one or the other until reverified.

*Delphinium scaposum* Greene

Mar. 3,  $\Delta = 1.57$ ,  $P = 18.9$

Tumamoc hill. The juice is very gelatinous.

*Diapedium torreyi* (A. Gray) Wootton and Standley

Apr. 8,  $\Delta = 1.61$ ,  $P = 19.3$

Among rocks on the north side of a small peak on Agua Verde creek. Cauline leaves, probably not fully matured, of plants in flower bud.

*Gnaphalium wrightii* A. Gray

Apr. 7,  $\Delta = 1.65$ ,  $P = 19.8$

Plants growing among the uppermost rocks of a small peak on Agua Verde creek. Age of leaves, which are covered with a dense wooly pubescence, undetermined, but probably of the previous fall.

*Nicotiana trigonophylla* Dunal

Apr. 7,  $\Delta = 1.30$ ,  $P = 15.7$

Growing in small honey-combed pockets on a rocky ledge. Agua Verde creek. Cauline leaves of flowering plants. In this locality the plants probably obtain an abundance of water during the rainy season but must be subjected to intense heat and drought during the drier periods.

*Pellaea wrightiana* Hook.

Mar. 20,  $\Delta = 1.28$ ,  $P = 15.4$

Old and young fronds from plants among rocks on the north side of Tumamoc hill.

*Pentstemon wrightii* Hook.

Apr. 7,  $\Delta = 1.40$ ,  $P = 16.9$

Cauline leaves of flowering plants. Agua Verde creek.

*Perezia wrightii* A. Gray  
Mar. 20,  $\Delta = 1.84$ ,  $P = 22.1$

In shady places among rocks on the north side near the top of Tumamoc hill. Large cauline leaves from plants in flower bud.

WINTER ANNUALS

*Amsinckia intermedia* F. and M.  
Mar. 20,  $\Delta = 1.08$ ,  $P = 13.0$

The species has been noted by Spalding [1909] as occurring chiefly in the shade, but he thinks moisture rather than light is the primary factor in determining its distribution. Thornber (in Spalding [1909], p. 105) states that, by virtue of a better developed root system than that of some other winter annuals, it is able to endure considerable drought, but is nevertheless of short duration.

Our samples were taken among rocks and in the shade of shrubs on the northern slope of Tumamoc hill. A first collection was made March 10, but was lost by the breaking of the tube. The collection on which this determination was based was secured on March 20, with much difficulty because so many of the plants had dried up.

*Astragalus nuttallianus* DC. var.  
Mar. 9,  $\Delta = 1.69$ ,  $P = 20.3$

Cannon [1911] (p. 31) describes this species as practically cosmopolitan in local distribution, occurring on all kinds of soil and exposures. Although our collection is assigned to the rocky slopes, the plants were taken only from the drier finer soil in the more open places. It did not seem to occur in the moistest places. Whole shoots except the flowers and pods, from Tumamoc hill.

*Calycoseris wrightii* A. Gray  
Mar. 20,  $\Delta = 1.12$ ,  $P = 13.5$

From Tumamoc hill: the juicy stems only could be secured.

*Chenopodium incanum* (Wats.) Heller  
Mar. 30,  $\Delta = 1.87$ ,  $P = 22.4$

Tumamoc hill, larger cauline leaves of young plants, about 3 inches high, which sprang up in great abundance after the last heavy spring rains.

*Daucus pusillus* Michx.  
Mar. 8,  $\Delta = 1.52$ ,  $P = 18.3$

This umbellifer is widely distributed in the Southwest, and has a rather extensive local distribution at Tucson. Cannon [1911] points out that the root system not only penetrates deeply but that it develops rapidly. Thus the species is ordinarily adapted to persist after the annuals with a more

superficial root system have disappeared. Our collections were made from vigorous plants growing in the shade of rocks, and apparently with an ample supply of moisture, on the north slope of Tumamoc hill. Whole shoots of plants which were for the most part not yet in flower.

*Eschscholzia mexicana* Greene

Mar. 8,  $\Delta = 1.22$ ,  $P = 14.7$

Deep gulch on Tumamoc hill leaves of large flowering plants.

*Eucrypta torreyi* (A. Gray) Heller

(1) Mar. 10,  $\Delta = 0.90$ ,  $P = 10.9$

(2) Mar. 27,  $\Delta = 0.91$ ,  $P = 11.0$

Leafy shoots of plants in flower and young fruit, growing in the most protected places among rocks on the northern exposure of Tumamoc hill.

*Mentzelia*, species undetermined

Mar. 3,  $\Delta = 1.39$ ,  $P = 16.8$

Plants from Tumamoc hill.

*Parietaria debilis* Forst.

Mar. 27,  $\Delta = 0.79$ ,  $P = 9.5$

A very delicate plant growing in the most protected and moistest spots on the northern slope of Tumamoc hill. Spalding [1909] (p. 20) and Cannon [1911] (p. 38) both point to this plant as one "possessing no characteristics which ally it to typical desert plants." Its water content is high. There are no especially developed means for the inhibition of transpiration. The root system is meager, with but slight vertical or horizontal penetration. It occurs almost exclusively on northern exposures in the shade of rocks where it has the maximum protection from insolation and aridity. When it occurs outside such habitats it is exceedingly dwarfed in size.

*Phacelia distans* Benth.

Mar. 3,  $\Delta = 1.07$ ,  $P = 15.2$

Leaves of plants in young fruit, Tumamoc hill.

*Senecio lemmoni* A. Gray

Mar. 20,  $\Delta = 1.18$ ,  $P = 14.2$

Cauline leaves from plants flowering in the more shaded places among rocks on the north slope of Tumamoc hill.

*Silene antirrhina* Linn.

Mar. 30,  $\Delta = 0.92$ ,  $P = 11.1$

Cauline leaves of flowering plants. East slope of Tumamoc hill.

*Streptanthus arizonicus* S. Wats.

Feb. 28,  $\Delta = 1.60$ ,  $P = 19.2$

Lower cauline leaves, either entire or deeply lobed, sometimes with distinctly purple hue, of flowering plants from Tumamoc hill.

*Thelypodium laciniatum* Greene

Mar. 30,  $\Delta = 1.74$ ,  $P = 20.9$

This determination was based on the upper cauline leaves of plants in mature or nearly mature fruit in shady spots on the north slope of Tumamoc hill. The plants had lost practically all their lower cauline leaves.

*Thysanocarpus laciniatus* var. *crenatus* Brewer

Mar. 9,  $\Delta = 1.10$ ,  $P = 13.2$

Tumamoc hill, leaves of flowering plants.

#### COMPARISONS OF CLIFFS AND ROCKY SLOPES WITH OTHER HABITATS

The differences between the rocky slopes and the foot-hills canyons have been sufficiently discussed under the preceding habitat. Comparisons with the bajada on the one hand and the arroyo on the other must now be taken up.

Consider first the species which occur in two or in all three of the habitats. The leaves of the shrub *Lippia wrightii*, the semi-succulent leaves of *Yucca macrocarpa*, the leaves of the winter annuals *Astragalus nuttallianus*, *Eschscholzia mexicana*, *Silene antirrhina* and *Streptanthus arizonicus*, and the stems of *Calycoseris wrightii* all show lower concentrations when growing in the arroyo than when on the rocky slopes or cliffs. *Thelypodium laciniatum* from the rocky ledges shows a higher concentration than *Thelypodium lasiophyllum* from the arroyo.

When examples of the same species growing in the arroyo and on the rocky slopes are compared, the plants of the arroyo show lower osmotic concentrations than those of the slopes, the only exception to this rule is furnished by a single collection of *Psilostrophe cooperi*. This was taken from a single plant, the vigorously growing shoots of which were not yet in flower, growing near the bottom of the west gulch of Tumamoc hill, where the plants apparently had an abundance of water from the higher ground. This collection, showing an osmotic concentration of 12.6 atmospheres, is compared with two others from the arroyo which gave freezing-point lowerings indicating pressures of 15.4 and 21.3. atmospheres. The lower of these was taken from the small, rocky Tumamoc arroyo, a habitat very similar to the one described above, but this collection occurred more than three weeks later in the season. The distinctly higher one was taken about two weeks later and from the margin of the lower portion of the large Tumamoc arroyo, in a place which might with

almost equal propriety be called mesa. Thus the single exception furnished by *Psilostrophe cooperi* cannot be given great weight.

On the other hand, the reader who compares the constants will observe that the differences in several of the cases are but slight. Thus no significance should be attached to such differences as those between the two collections of *Silene antirrhina*. *Thelypodium laciniatum* from the rocky cliffs was taken in nearly ripe fruit about three weeks after *T. lasiophyllum* from the arroyo, where it was found at about the height of its flowering period. The lower leaves of the plants from the rocky slopes had fallen, and the cauline leaves from the upper portion of the stem fell rather readily, although for the most part quite green.

Although the indications of these few cases in which it was possible to compare species from the rocky ledges and the arroyo are that the plants in the former have a higher osmotic pressure than those of the latter, we believe the evidence is not conclusive.

Turning now to a comparison of the rocky slopes with the bajada slopes we note that the two determinations of the sap concentration of the dominant desert half-shrub *Gaertneria deltoides*, made from material collected on the dry bajada slopes, are each higher than the one from the rocky ground southeast of the laboratory building. It must be noted, however, that considerable rain had fallen between the first collection from the dry slopes of Tumamoc, which gave the highest osmotic value, and the last one from the moister portion of the hill back of the Laboratory. This may be a disturbing factor. In short, the difference between the two habitats may be due merely to the occurrence of precipitation between the times of the two collections.

The two determinations for the sap of *Psilostrophe cooperi* from the mesa or bajada are each distinctly higher than the one from the rocky slope. The latter is, however, as has been pointed out above, hardly typical.

The perennial herb *Bahia absinthifolia* var. *dealbata* shows a far lower concentration on Tumamoc hill than on an Agua Verde creek bajada.

The perennial herb *Nicotiana trigonophylla* shows a slightly higher osmotic concentration in the single locality examined for the rocky slopes than in the one examined for the mesa. There is, however, some question whether these particular localities are not rather anomalous for the habitats to which they have been assigned. It is very probable that the mesa locality is far better supplied with water than is usually the case, and that the rocky slope where the collection was made is subjected to great dryness during portions of the year.

The annual herb *Eschscholzia mexicana* shows distinctly higher sap concentration when growing on the mesa or bajada slope. The winter annual *Streptanthus arizonicus* also shows a higher, though probably only insignificantly higher, concentration.

## C. THE BAJADAS OR MESA-LIKE SLOPES

The bajadas are the more gently sloping masses of detrital materials, the last product, except for the flood plain, of the weathering down of the mountains. So nearly level do they appear in their lower stretches when casually viewed that the term mesa is popularly applied to them. They are traversed by the arroyos or washes, which may have a general course with the prevalent slope where the gradient is pronounced. Where the slope is gentler and the extent great, cross channels are developed. These factors make for considerable topographic and edaphic diversity. Near the base of the mountains, rocks, gravel and sand are mixed with the fine adobe clay. At the terminus of the slope, only the finer soil particles have been deposited.

Because of its vast extent and diversified nature, an account of the soil moisture of these gentler detrital slopes is almost impossible. Such moisture determinations as we have are those made by Livingston, Spalding, and Cannon in the immediate vicinity of the Desert Laboratory. Livingston (in Spalding [1909], p. 86 *et seq.*) furnishes evidence that these soils are considerably drier than those of Tumamoc hill. Further proof of this is furnished by Cannon [1911] (pp. 17-18) who found that during the period studied by him the water content of the soil of the bajada slope of the Laboratory domain was always lower than that of the hill. For a depth of 20 cm. the soil of the bajada slope is in many localities air dry throughout the greater part of the year. The brevity of the period during which the soil of the mesa contains water sufficient for the growth of annuals or of perennials without either a water storage system or very deeply penetrating roots is one of the factors which contribute to render it the most extremely xerophytic of any of the habitats studied.

There seems serious danger in generalizing the observations which have been made on but a limited area and applying them without qualification to the wider region over which our collections were made. Nevertheless, there seems little doubt that the soil of the gentler slopes is permanently far dryer than that of the more precipitous rocky ones, although the former are irrigated by the superficial run-off from the higher levels, and after heavy rains may be very wet indeed. The mesa slopes are to a considerable extent underlaid by caliche at no great depth. This prevents the storage of moisture at greater depth and facilitates its loss by sub-surface run-off. The soil is usually much coarser, because of the mixture of sand and finely broken rock fragments. Thus, water-holding capacity is low and the possibility of loss by evaporation very great.

The difference between the bajada or mesa-like slopes and the cliffs and rocky slopes seems to us to be brought out in a significant manner by a slight rearrangement of Thornber's data for this area (in Spalding [1909], p. 104) which we present here as table I.

Taking all the suffrutescent to arborescent perennials together, we note that the natural flora of the hill is far in excess of that of the mesa-like slope or of the Santa Cruz flood plain. The flood plain is slightly, perhaps not significantly, richer in species than the mesa. For perennial and biennial herbs the same condition prevails, except that the superiority of the flood plain over the bajada is even more pronounced in this case.

Combining the herbaceous and larger perennials, a distribution which is distinctly bimodal is obtained: the relatively rich perennial flora of the hill and of the river bottom are separated by a zone apparently poorly suited for

TABLE I

*Number of species of various vegetation forms occurring in each of three different habitats (Rearranged from Thorner's data)*

VEGETATION FORMS	HABITAT:			TOTALS
	Tumamoc hill	Mesa-like slopes	Santa Cruz flood plain	
	<i>species</i>	<i>species</i>	<i>species</i>	<i>species</i>
Trees.....	2	2	11	15
Shrubs.....	16	10	10	36
Woody twiners.....	2	—	3	5
Dwarf shrubs.....	13	4	—	17
Half-shrubs.....	21	7	3	31
All ligneous perennials.....	54	23	27	104
Perennial and biennial herbs.....	39	25	34	98
All perennials.....	93	48	61	202
Annual herbs				
Long lived.....	—	9	28	37
Winter annuals.....	38	46	16	100
Summer annuals.....	7	25	12	44
All annuals.....	45	80	56	181
Totals.....	138	128	117	383

the growth of long-lived species. That the smaller number of species on the mesa is not due to its more limited area or to some other condition making for greater taxonomic poverty is clearly shown by the data for the annuals, of which 80 species occur on the mesa as compared with 45 on the hill and 56 on the flood plain. If the long-lived annuals which occur chiefly on the flood plain be left out of account we find that for both winter and summer annuals the mesa has about as many species as the two other habitats together.

The explanation of this fact of local distribution is, it seems to us, that the mesa is supplied with ample water for the development of the rapidly matur-

ing annuals of both winter and summer but presents conditions far too severe for any but the hardiest perennials.

The cryoscopic constants for the individual species will now be considered.

#### TREES AND SHRUBS

*Agave palmeri* Engelm.

Apr. 8,  $\Delta = 0.92$ ,  $P = 11.1$

Leaves of a vigorous plant in vegetative condition; Agua Verde creek.

*Atriplex canescens* James

Apr. 6,  $\Delta = 4.08$ ,  $P = 48.8$

One or two very old specimens were seen on the edge of a low bluff on Agua Verde creek. Here they seemed to be in a habitat fundamentally different from the salt spots in which the species is usually found. Leaves as nearly as possible of the spring growth

*Covillea glutinosa* (Engelm.) Rydb.

(1) Tumamoc hill, Mar. 21,  $\Delta = 2.79$ ,  $P = 33.5$

(2) Tumamoc hill, Mar. 30,  $\Delta = 2.18$ ,  $P = 26.2$

(3) Agua Verde creek, Apr. 8,  $\Delta = 2.85$ ,  $P = 34.2$

The creosote bush has been so extensively observed and discussed by those who have worked in the southwestern deserts that a detailed summary of the literature in this place is unfeasible. Spalding [1904] made detailed observations on the water relations of plants in the neighborhood of the Desert Laboratory. Again [1909] (pp. 16–17, 30–31, 36–37) he discusses local distribution on Tumamoc hill, and in a chapter contributed to this volume (*loc. cit.*, p. 86–87) Livingston treats the soils of the *Larrea* (= *Covillea*) slopes. Cannon [1911] and MacDougal and Cannon [1910] treat the root habits.

That *Covillea* is one of the most successful of the desert perennials is obvious from the fact that it is the dominant plant over thousands of square miles of territory in the arid southwest, and botanists have sometimes called the type of habitat which we are here considering “the *Larrea* slopes.”

Our first collection from Tumamoc hill was taken when the plants had practically ceased flowering. The foliage—all or practically all of the previous year's growth—was not in the best condition, probably because of a considerable dry period, which had just been broken. The effect of these rains was soon apparent in the appearance of the foliage, and is also, we believe, to be seen in the distinctly lower osmotic concentration of the leaf sap taken nine days later. The condition of the plants from the *Mortonia* habitat on Agua Verde creek was very similar to that prevailing among the individuals from which the Tumamoc collections were made. The variation in the concentration of the sap of the leaf is in good agreement with the results for absorption of water obtained by Spalding [1906]. Probably the determinations here given are more nearly minimum than maximum values.

*Mortonia scabrella* A. Gray(1) Mar. 25,  $\Delta = 4.78$ ,  $P = 57.2$ (2) Apr. 8,  $\Delta = 4.32$ ,  $P = 51.7$ 

*Mortonia* is a shrub which Dr. Shreve tells us is strictly limited to limestone areas, at least in the southern Arizona deserts. The leaves are small, very hard and have a remarkable duration on the plant—sometimes lasting as long as six or seven years. Our collections were made from a gentle northern slope on Agua Verde creek, where *Mortonia*, *Covillea*, *Dasylirion* and *Agave* were the chief plants—*Mortonia* being the dominant form. Here the large plants, say those of a meter and more in height, must be very old. Growing among the larger plants were numerous smaller ones from 10 to 35 cm. in height, bearing leaves which were apparently not so dry as those on the larger plants. That these plants were in a somewhat different condition with regard to water supply, to their capacity for absorbing water or to the relative amount retained is evidenced by the fact that on March 25 some of the young plants were beginning to send out tender green shoots, while on April 8 this growth had progressed so far that it was possible to collect a sample of the leaves of the spring growth. Such growth was, however, by no means universal. Probably not more than 1 in 50 or 1 in 100 of the young plants showed such activity. *On the old plants not a single instance of growth could be seen.*

From the leaves of the older plants we were unable to obtain any juice at all. Hardly enough fluid could be pressed from a mass of the frozen and ground leaves to moisten the cloth in which they were wrapped.

We had hoped that it would be possible to collect the leaves for the several years' growth and to determine the concentration of the sap of those of each age separately. This proved quite impossible. Because of the great labor of collecting samples large enough for extractions of sap, the time required would have been more than we had available. Furthermore the fact that a small proportion of the shoots were making an apparently normal spring growth at the time when our collections were made renders it quite probable that, in at least a sensible number of cases, consecutive zones of mature leaves do not represent consecutive years.

A volume of about 900 cc. of tightly packed leaves from the small plants, which must themselves be many years old, gave, after being frozen and ground in a meat chopper, a mass which has about the consistency of moist sawdust. This was placed in the bowl of the large press and after about eight hours' squeezing at the highest pressure possible with frequent rearrangement of the sample, about 17 cc. of exceedingly viscous, almost black juice, which gave practically no sediment on centrifuging, was obtained.

A rain had apparently intervened between our first and second visit, the juice from the mature leaves was obtained more readily in the second case, and was less concentrated. It was also possible to obtain a sample of the young leaves. These were still flat instead of saddle-shaped, and tender

and green, instead of grey and with the characteristic whitish margins. The juice was extracted readily from these and gave:  $\Delta = 1.95$ ,  $P = 23.4$ .

*Olneya tesota* A. Gray

Apr. 12,  $\Delta = 2.47$ ,  $P = 29.7$

Leaves of the previous year's growth, apparently in a functioning condition, were collected from small trees in Robles pass.

*Prosopis velutina* Wooton

(1) Near mouth of Pima canyon, Apr. 3,  $\Delta = 2.08$ ,  $P = 25.0$

(2) In rocky parts of Robles pass, Apr. 12,  $\Delta = 2.33$ ,  $P = 27.9$

Leaves of the spring's growth from flowering plants, the collections from Robles pass were made from very dwarfed, shrub-like trees.

*Yucca elata* Engelm.

Mar. 25,  $\Delta = 3.04$ ,  $P = 37.4$

The plants grew near the Agua Verde creek arroyo. The leaves are very narrow, hard and fibrous. Juice could be obtained only by reducing the leaves to a pulp on an anvil before freezing and squeezing. Immature leaves from the centre of the plant gave a larger quantity of juice and a lower osmotic value:  $\Delta = 2.42$ ,  $P = 29.0$ . Because of the various difficulties in collecting and working up this species, the determinations must be looked upon as approximate only.

#### DWARF SHRUBS, HALF-SHRUBS AND WOODY TWINERS

*Gaertneria deltoidea* (Torr.) Kuntze

(1) Tumamoc hill slopes, Mar. 6,  $\Delta = 2.23$ ,  $P = 26.7$

(2) Santa Catalina bajada, Apr. 3,  $\Delta = 1.98$ ,  $P = 23.8$

While one determination for this species has been given for the rocky slopes, its typical habitat is unquestionably the mesa. Spalding in his ecological scheme [1909] (pp. 16-17) has divided the mesa-like slopes into the creosote bush association and the *Franseria* (= *Gaertneria*) association. It is perhaps safe to say that in easy striking distance of the Desert Laboratory there are hundreds of square miles of desert in which this is the dominant species. On the Santa Catalina slopes, between the mouth of Pima canyon and the Rillito, *Gaertneria* is one of the most conspicuous species. Here it may occur with the mesquite, palo verde, ocatillo, and giant cactus and the two or three frequent arborescent opuntias known as chollas, or it may be almost the only shrubby species over large areas. In such places it occurs with the regularity of spacing that characterizes the creosote bush when growing in dry localities. It begins to be seen as one leaves the Rillito bottoms and practically disappears on the steeper, rockier terrain below the mouth of the can-

yon. In the canyon itself it is almost entirely replaced by *Gutierrezia ambrosioides* and *G. cordifolia*.

Further information concerning this species may be obtained from Cannon's paper on the root habits of desert plants, and from Lovejoy's [1913] detailed biological study.

Leaves of flowering shoots were used in our determinations.

*Perezia nana* A. Gray

Mar. 23,  $\Delta = 1.63$ ,  $P = 19.6$

Sterile, stony soil on the edge of the *Mortonia* tract, Agua Verde creek. Leaves of plants not yet in flower.

*Psilostrophe cooperi* (A. Gray) Greene

(1) Mar. 25,  $\Delta = 1.84$ ,  $P = 22.2$

(2) Apr. 7,  $\Delta = 1.90$ ,  $P = 22.9$

Both collections were made near Agua Verde creek. The plants were in young bud or flowering.

*Thymophylla acerosa* new comb.

Apr. 7,  $\Delta = 1.75$ ,  $P = 21.1$

*Thymophylla hartwegi* (A. Gray) Wootton and Standley

Apr. 7,  $\Delta = 2.26$ ,  $P = 27.1$

These two species occurred practically together, in the most open spots on the more finely disintegrated rock of the slopes near Agua Verde creek. Both were in full flower when collected. The subulate leaves are very small and the collection of samples was exceedingly laborious. Possibly the lower value for *T. acerosa* may be due to a relatively larger proportion of leaves from recently grown shoots.

#### PERENNIAL HERBS

*Bahia absinthifolia* var. *dealbata* A. Gray

Apr. 7,  $\Delta = 1.91$ ,  $P = 23.0$

Cauline leaves, chiefly from flowering plants, collected near the *Thymophylla* species, Agua Verde creek.

*Lesquerella purpurea* (A. Gray) S. Wats.

Mar. 26,  $\Delta = 1.72$ ,  $P = 20.7$

Leaves of flowering plants growing with *Yucca*, *Agave* and *Dasylirion*, in very sterile rocky localities, Agua Verde creek.

*Melampodium leucanthum* Torr. and Gray

Apr. 7,  $\Delta = 1.73$ ,  $P = 20.8$

Agua Verde creek, leaves of flowering plants, occurring chiefly in the shade of shrubs or rocks.

*Nicotiana trigonophylla* DunalApr. 3,  $\Delta = 1.23$ ,  $P = 14.8$ 

Cauline leaves of flowering plants, from near the mouth of one of the larger arroyos emptying into the Rillito. Probably the locality had far more soil moisture than most places classified as mesa.

*Thamnosma texana* Torr.Mar. 23,  $\Delta = 1.59$ ,  $P = 19.1$ 

Occurs with the *Lesquerella* entered above. The whole shoots, excepting the flowers, were taken.

## WINTER ANNUALS

*Eriophyllum lanatum* (Pursh) Forbes(1) Mar. 8,  $\Delta = 1.95$ ,  $P = 23.4$ (2) Mar. 16,  $\Delta = 1.96$ ,  $P = 23.6$ 

This minute composite apparently grows in the driest places. Frequently there is only a single stem terminated by the relatively large head; occasionally there may be four or five branches from the base. Even when the whole plant, excepting the roots, was taken the securing of samples was exceedingly laborious.

*Eschscholzia mexicana* Greene(1) Tumamoc hill, Mar. 3,  $\Delta = 1.62$ ,  $P = 19.5$ (2) Tumamoc hill, Mar. 6,  $\Delta = 1.92$ ,  $P = 23.0$ 

Leaves of flowering plants were used.

*Lepidium lasiocarpum* Nutt.Mar. 8 and 10,  $\Delta = 2.08$ ,  $P = 24.9$ 

From Tumamoc hill, it was necessary to take the whole plant with the exception of the larger stems and the somewhat dried or dusty rosette leaves, since the cauline leaves were very small. Probably photosynthesis was to a considerable extent performed by the numerous green fruits.

Wootton and Standley [1915] give the New Mexican habitat of this species as dry fields in the Lower and Upper Sonoran zone, and state that it is often a weed in irrigated land. That it is able to penetrate into at least slightly developed salt spots is shown by the fact that it was found growing with *Atriplex canescens angustifolia* mentioned later in this paper.

*Nemoseris neo-mexicana* (A. Gray) GreeneMar. 4,  $\Delta = 1.17$ ,  $P = 14.1$ 

Tumamoc hill, rosette and cauline leaves. These were in a rather unsatisfactory state. The plants which grew in the driest places were badly dried, while those which were in spots where there was more moisture can hardly

be considered typical of the habitat to which they have been assigned. The leaves of this species are of a rather ephemeral type and soon dry up, leaving only the juicy stems and flowers. Two determinations on the sap of the tender stems were made. The one taken at the same time as the leaves gave:  $\Delta = 1.25$ ,  $P = 15.0$ . These values are slightly higher than those given for the leaves gathered at the same time, but the difficulties in getting really typical samples are very great and the difference may not mean that in the same plant the juice of the stems is more concentrated than that of the leaves. Indeed another determination made on the juice of stems collected March 20 gave lower values, i.e.:  $\Delta = 1.12$ ,  $P = 13.5$ .

*Streptanthus arizonicus* S. Wats.

Mar. 23,  $\Delta = 1.61$ ,  $P = 19.4$

Leaves of very dwarfed plants in early flower occurring largely in the shade of *Mortonia* or *Yucca* on Agua Verde creek.

#### COMPARISONS OF THE BAJADAS WITH OTHER HABITATS

Comparisons of the plants of the *Covillea* slopes, as they have sometimes been called, with the foot-hills canyons and with the rocky cliffs have already been made in the sections devoted to those habitats.

The half shrub *Psilostrophe cooperi* shows slightly higher concentration on the mesa than in the arroyo. The winter annuals *Eschscholzia mexicana*, *Nemoseris neo-mexicana* and *Streptanthus arizonicus* all show far higher concentrations when growing on the bajada slopes than when in the arroyo.

Only one species from the bajada was also secured from the salt spots. One determination on a single very large specimen of *Atriplex canescens*, growing on a bank above the bed of Agua Verde creek, gave a concentration intermediate between two determinations for this species from different salt spots.

#### D. THE ARROYO OR WASH

A most characteristic feature of the southwestern deserts is the arroyo or wash. When typically developed, as they are when traversing the bajada or mesa-like slopes, these arroyos are characterized superficially by a flat bed of coarse sand and gravel almost free of vegetation, with low-lying banks of mixed stones, gravel, sand and adobe, often cut into secondary channels covered with a tangle of preponderantly arborescent vegetation.

The sand of the bed and the soil of the low banks are very deep. The torrents of water of the violent summer rains sink quickly into the bed, while the lighter showers are of but little consequence in rendering the soil suitable for plant growth. In the spring, however, the presence of a number of annuals growing along the edges of the drifts of finer soil evidences considerable soil moisture. The deeper-rooted semi-arborescent plants which fringe the edges

of these dry water courses with a thorny thicket are probably in many instances able to reach a more permanent supply of water.

In the process of base leveling these arroyos are gradually extending their way into the steeper slopes, where they terminate in rocky gullies, with little sand or even coarse gravel in the bottom. Thus if the arroyo be followed for its whole length, it shows all transition stages from flood plain to outwash slope and rocky cliffs.

The collections from arroyos were taken from the large "west wash" on the Desert Laboratory domain and from the dry bed of Agua Verde creek in the Rincon mountains. Both these are typical sandy arroyos, but differ considerably in the character of the surrounding topography and in elevation. In addition a number of collections were made from a smaller and far more rocky gully which draws its water, when it carries any, in part from the eastern exposure of Tumamoc hill. Except in the lowest portion of its course it is hardly a typical arroyo, but such collections as we have made from there have been classified with those from the west wash and from Agua Verde creek.<sup>4</sup> Possibly, it might be desirable to differentiate the constants from these two types of localities, but this is hardly allowable on the basis of the data at present available.

#### TREES AND SHRUBS

*Acacia constricta paucispina* Wooton and Standley

Apr. 1,  $\Delta = 1.46$ ,  $P = 17.5$

Young leaves, from Tumamoc hill.

*Celtis reticulata* Torr.

Apr. 6,  $\Delta = 1.62$ ,  $P = 19.4$

Young but apparently full grown leaves from a single tree, in flower on the bank of Agua Verde creek.

*Fraxinus toumeyi* Britt. and Shaf.

(1) Mar. 24,  $\Delta = 1.39$ ,  $P = 16.7$

(2) Apr. 8,  $\Delta = 1.44$ ,  $P = 17.3$

(3) Apr. 8,  $\Delta = 1.63$ ,  $P = 19.6$

These determinations were made from two trees, one in the bed, the other on the edge of Agua Verde creek. The first collection, March 24, was made when the leaves were very young, but the second from the same tree, taken April 8 when the leaves were practically fully grown but not yet quite mature, gives practically the same constant. A collection from another tree on April 8 gave distinctly higher values than either of those from the first individual.

<sup>4</sup> Agua Verde creek has been used in this paper to designate a considerable area of rocky cliffs, mesa and arroyo. The term refers specifically to the arroyo proper only when this is clearly indicated by the context.

*Lippia wrightii* A. Gray

Apr. 1,  $\Delta = 1.26$ ,  $P = 15.1$

This shrub is abundant on the rocky slope of Tumamoc hill but not common in the arroyo, where this sample was taken from the young shoots of vigorous shrubs.

*Yucca macrocarpa* (Torr.) Coville

Mar. 25,  $\Delta = 1.53$ ,  $P = 18.4$

From Agua Verde creek.

#### DWARF SHRUBS, HALF-SHRUBS AND WOODY TWINERS

*Menodora scabra* A. Gray

Apr. 1,  $\Delta = 1.34$ ,  $P = 16.1$

Leaves chiefly from flowering shoots, Tumamoc hill.

*Psilostrophe cooperi* (A. Gray) Greene

(1) Tumamoc hill, Mar. 21,  $\Delta = 1.78$ ,  $P = 21.3$

(2) Tumamoc hill, Mar. 30,  $\Delta = 1.28$ ,  $P = 15.4$

Both collections were made from flowering plants. The second was made from the small rocky arroyo, while the first, giving the greater depression of the freezing point, was taken near the lower course of the large arroyo, in a locality which might with almost equal propriety have been assigned to the mesa.

*Senecio filifolius* Nutt.

Mar. 25,  $\Delta = 1.12$ ,  $P = 13.5$

A single plant, not in flower, growing in the sandy bed of Agua Verde creek.

#### PERENNIAL HERBS

*Astragalus allochorus* A. Gray

Mar. 24,  $\Delta = 1.19$ ,  $P = 14.3$

Leaves of plants, in flower and young fruit, forming mats on the gravelly, sandy bed of Agua Verde creek.

*Pentstemon spectabilis* Thurber

Apr. 6,  $\Delta = 1.41$ ,  $P = 17.0$

A single plant, in flower, found among drift on the edge of Agua Verde creek arroyo.

*Rumex hymenosepalus* Torr.

(1) Mar. 25,  $\Delta = 1.01$ ,  $P = 12.1$

(2) Apr. 8,  $\Delta = 0.90$ ,  $P = 10.9$

This species (which has large underground organs) has leaves of about the size of the larger mesophytic species of the genus. It is rather closely con-

finer in its distribution to the deep sandy drifts along the arroyos. Our collections were made from flowering plants on Agua Verde creek.

WINTER ANNUALS

*Astragalus nuttallianus* DC. var.

Mar. 16,  $\Delta = 1.34$ ,  $P = 16.1$

Small shoots from flowering plants, on Tumamoc hill.

*Calycoseris wrightii* A. Gray

(1) Mar. 17,  $\Delta = 0.97$ ,  $P = 11.6$

(2) Mar. 30,  $\Delta = 0.76$ ,  $P = 9.1$

Both collections were made from about the same spot in the small arroyo, Tumamoc hill. The first comprised chiefly rosette leaves, the latter contained a far larger proportion of cauline leaves. The lower concentration found on March 30 is probably due to the considerable rains which fell between the dates of the two collections and gave a vigorous growth to all the arroyo vegetation.

A sample of sap extracted from the stems on March 17 gave almost exactly the same constants as did that from the leaves, *i.e.*:  $\Delta = 0.98$ ,  $P = 11.8$ .

*Calyptridium monandrum* Nutt.

(1) Mar. 7,  $\Delta = 0.86$ ,  $P = 10.4$

(2) Mar. 21,  $\Delta = 0.69$ ,  $P = 8.3$

The succulent strap-shaped leaves form a flat rosette on the sand. Both lots were collected in the large arroyo, Tumamoc. In the second collection the flowering shoots were far more developed.

*Eriogonum pusillum* Torr. and Gray

Mar. 8,  $\Delta = 1.23$ ,  $P = 14.8$

*Eriogonum aberteanum* Torr.

Mar. 21,  $\Delta = 1.32$ ,  $P = 15.8$

*Eriogonum deflexum* Torr.

Mar. 10,  $\Delta = 1.31$ ,  $P = 15.7$

These three species, all from the lower course of the large arroyo on the Laboratory domain, are not strictly speaking winter annuals but are classified by Thornber as long lived annuals. All three determinations are based on the rosette leaves of plants just beginning to send up flowering shoots.

*Eschscholzia mexicana* Greene

(1) Tumamoc hill, Mar. 3,  $\Delta = 1.13$ ,  $P = 13.6$

(2) Tumamoc hill, Mar. 30,  $\Delta = 0.99$ ,  $P = 11.9$

*Eulobus californicus* Nutt.Apr. 1,  $\Delta = 0.89$ ,  $P = 10.7$ 

Leaves of plants in young fruit, Tumamoc hill. The juice from the rather tender stems of the same individuals gave somewhat lower constants:  $\Delta = 0.72$ ,  $P = 8.7$ .

*Gilia floccosa* A. Gray(1) Tumamoc arroyo, Mar. 8,  $\Delta = 1.25$ ,  $P = 15.1$ (2) Agua Verde creek, Mar. 24,  $\Delta = 0.93$ ,  $P = 11.2$ 

Thornber describes this as one of the winter annuals which, by means of a better developed root system, is able to endure considerable drought, but is nevertheless a species of but short duration. Because of the minute size of the leaves the whole shoot, of plants in flower or young fruit, was taken.

*Gilia inconspicua* (Smith) Dougl.Mar. 24,  $\Delta = 0.96$ ,  $P = 11.5$ 

The rosette and cauline leaves were taken from fruiting plants in the bed of Agua Verde creek.

*Lappula occidentalis* (S. Wats.) GreeneTumamoc, Mar. 8,  $\Delta = 1.48$ ,  $P = 17.8$ *Lepidium*, species undeterminedTumamoc hill, Mar. 16,  $\Delta = 1.17$ ,  $P = 14.1$ *Lepidium medium* Greene(1) Mar. 25,  $\Delta = 1.12$ ,  $P = 13.5$ (2) Apr. 8,  $\Delta = 1.21$ ,  $P = 14.6$ 

On gravelly drifts in the bed of Agua Verde creek; younger stems, leaves and inflorescences.

*Lepidium thurberi* WootonMar. 24,  $\Delta = 1.13$ ,  $P = 13.6$ 

On gravelly drifts in the bed of Agua Verde creek. Rosette and cauline leaves of plants just coming into flower. Note the excellent agreement of these four determinations from arroyos. They give only about half the depression of the freezing point obtained for *L. lasiocarpum* from the mesa.

*Lesquerella gordonii* (A. Gray) S. Wats.(1) Tumamoc arroyo, Feb. 28,  $\Delta = 1.26$ ,  $P = 15.1$ (2) Agua Verde creek, Mar. 24,  $\Delta = 1.22$ ,  $P = 14.7$ 

Leaves of flowering plants. *L. gordonii* is a widely distributed winter annual, which, in its local occurrence, is by no means limited to the arroyos, although it must be pointed out that on Agua Verde creek it seemed to be replaced on the dry rocky slopes by the perennial species *L. purpurea*. It may

even penetrate into the less developed salt spots, as is shown by the fact that it was taken with the *Atriplex canescens angustifolia* to be considered below.

*Lupinus micensis* Jones

Mar. 24,  $\Delta = 0.85$ ,  $P = 10.2$

Apr. 6,  $\Delta = 0.91$ ,  $P = 11.0$

Leaves of small plants, in flower or fruit, growing in the sandy bottom of Agua Verde creek. In Pima canyon, growing in soil, the plant attains a far greater size. Unfortunately our tubes from that locality were broken.

*Lupinus sparsiflorus* Benth.

(1) Tumamoc arroyo, Mar. 10,  $\Delta = 0.96$ ,  $P = 11.5$

(2) Tumamoc arroyo, Mar. 30,  $\Delta = 0.99$ ,  $P = 11.9$

(3) Agua Verde creek, Mar. 24,  $\Delta = 0.97$ ,  $P = 11.7$

(4) Agua Verde creek, Apr. 6,  $\Delta = 0.88$ ,  $P = 10.5$

Leaves of plants in flower or bearing young fruit.

*Nemoseris neo-mexicana* (A. Gray) Kuntze

(1) Mar. 17,  $\Delta = 0.85$ ,  $P = 10.2$

(2) Mar. 30,  $\Delta = 0.74$ ,  $P = 8.9$

Rosette and cauline leaves. A determination on the juice of the stems of the first collection gave:  $\Delta = 0.92$ ,  $P = 11.1$ . Compare the notes under *Calycoseris*, above.

*Oenothera caespitosa* Nutt.

Mar. 2,  $\Delta = 1.07$ ,  $P = 12.8$

Leaves of flowering rosettes, Tumamoc hill.

*Silene antirrhina* Linn.

Apr. 1,  $\Delta = 0.92$ ,  $P = 11.0$

From Tumamoc hill, leaves of plants in young fruit.

*Sophia pinnata* (Walt.) Howell

Mar. 7,  $\Delta = 1.47$ ,  $P = 17.7$

Larger cauline leaves of plants in young fruit. Lower portion of large arroyo, where it merges into the mesa, Tumamoc hill.

*Sphaerostigma chamaenerioides* (A. Gray) Small

(1) Tumamoc arroyo, Mar. 21,  $\Delta = 0.96$ ,  $P = 11.5$

(2) Agua Verde creek, Mar. 24 and 25,  $\Delta = 0.96$ ,  $P = 11.6$

The stems of the fruiting plants on Tumamoc hill, from which the cauline leaves were collected, were also saved and gave:  $\Delta = 0.94$ ,  $P = 11.3$ . These values do not differ significantly from those for the leaves. Because of the difficulties of collecting and the agreement between the sap from the

stems and the leaves demonstrated for the Tumamoc collection of this species, the whole shoot was taken in the case of the Agua Verde creek collection.

*Streptanthus arizonicus* S. Wats.

(1) Feb. 28,  $\Delta = 1.17$ ,  $P = 14.1$

(2) Mar. 21,  $\Delta = 1.22$ ,  $P = 14.7$

The collections, both from Tumamoc hill but on different dates and from different arroyos, are in excellent agreement. A sample from the stems of the second collection gave:  $\Delta = 1.40$ ,  $P = 16.9$ .

*Thelypodium lasiophyllum* Greene

Feb. 28,  $\Delta = 1.63$ ,  $P = 19.6$

Leaves of flowering plants, Tumamoc hill. While we have a freezing-point lowering for this one sample from the arroyo only, the species is not confined to the arroyo. It may occur in at least slightly developed salt spots, as is shown by its occurrence with *Atriplex canescens angustifolia* (see below).

#### E. SALT SPOTS

The salt spots of the region under consideration are low, imperfectly drained areas in which there has been a pronounced accumulation of alkaline salts, especially those of sodium. We are indebted to Cannon [1913] for the systematic study of the vegetation of these spots. Spalding [1909] (pp. 13-14) also sketches their chief characteristics. Biologically, they are characterized not only by the presence of several species of Chenopodiaceae, but by the absence of the great majority of the species of the surrounding area.

It is a matter of great regret that the freezing point of the juices of some of the less typically halophytic species, which, nevertheless, penetrate to some distance into the outer zones of the salt spots, could not be taken up. Our collections came in part from the spot originally studied by Cannon; but a systematic series of collections made from the various zones described by him in his interesting paper was precluded by the fact that the particular spot studied by him had since been burnt off. Other series were taken on the road to San Xavier Mission and one sample from a slightly developed spot on the road from Tucson to Vail about five miles out of the city.

#### TREES AND SHRUBS

*Atriplex canescens* (Pursh) James

(1) Apr. 4,  $\Delta = 5.65$ ,  $P = 67.5$

(2) Apr. 12,  $\Delta = 3.30$ ,  $P = 39.5$

The first of these was taken from Dr. Cannon's salt spot. Here the plants were very dwarfed indeed. The second collection, from near San Xavier Mission, was from larger shrubs. In the neighborhood of the Mission these shrubs do not penetrate into the most alkaline places.

*Atriplex canescens* is a species widely distributed in the west and southwest. The floras describe it as occurring in alkaline spots, in arroyos and on dry mesas. Wootton and Standley and Thornber state that, while the species tolerates large quantities of alkali, it also occurs where there is little or none. Cannon and Spalding note that in the zonal disposition of the vegetation of the salt spot *A. canescens* is one of the outermost species.

*Atriplex canescens* var. *angustifolia* Cockerell  
 $\Delta = 2.73, P = 32.8$

In a depression on the mesa, about five miles from Tucson on the Vail road. The locality while evidently alkaline was not a highly developed salt spot, as shown by the presence of *Corillea* and several winter annuals; e.g., *Lepidium lasiocarpum* Nutt., *Lesquerella gordonii* (Gray) Wats., *Sophia halictorum* Cockerell, *Phacelia arizonica* Gray, and *Thelypodium lasiophyllum* Greene.

*Atriplex polycarpa* S. Wats.  
 Apr. 4,  $\Delta = 4.35, P = 52.0$

Leaves of shrubs not in flower, from Dr. Cannon's salt spot.

#### DWARF SHRUBS AND HALF-SHRUBS

*Dondia moquini* (Torr.) Nelson  
 Apr. 4,  $\Delta = 2.85, P = 34.2$

Young shoots from the old wood, Dr. Cannon's salt spot.

#### WINTER ANNUALS

*Atriplex fasciculata* S. Wats.  
 (1) Apr. 12,  $\Delta = 2.94, P = 35.3$   
 (2) Apr. 12,  $\Delta = 1.60, P = 19.3$

Near San Xavier Mission, see discussion below.

*Monolepis nuttalliana* (R. and S.) Engelm.  
 Apr. 12,  $\Delta = 1.34, P = 16.1$

Near San Xavier Mission, see discussion below.

As might have been expected, the highest concentrations found in any habitat occur on the salt spots. Altogether only eight determinations are available. These average 37.1 atmospheres. The shrubs average higher than the annuals, but the number of observations is too few to attach any final significance to the result in this instance.

Both ligneous perennials and annuals show great variation within the species. Thus *Atriplex canescens* growing in the salt spot studied by Cannon [1913] shows an osmotic value not far from seventy-five per cent. higher than the same species collected near the San Xavier Mission. An old shrub on an overhanging bank of Agua Verde creek, assigned to the mesa, gave an

intermediate value. The variety *angustifolia* of this species gives the lowest concentration of the four determined.

All of these salt bushes offer great difficulties to the extraction of their sap. The determinations have therefore a larger possible error than in the case of many other species.

The succulent-leaved half-shrub *Dondia moquini*, which has been observed by Cannon and Spalding to be associated with species of *Atriplex* in the innermost vegetation zones of the salt spots, shows a pressure of the same order as the sclerophyllous halophytes.

*Atriplex fasciculata*, an annual, is particularly interesting. It occurred almost alone in the lowest parts of the salt spot on the San Xavier Mission road. Here it was very dwarfed, and its juice had a concentration of 35 atmospheres. In this same spot it was found growing luxuriantly in a pit where it seems quite probable that the salts in the soil would be leached out and carried away underground. Here it gave only about half the depression of the freezing point that it did when growing on the flat alkali surface.

The great differences here recorded show the rather remarkable power of accommodation exhibited by halophytic species—a power which has been observed by a number of writers.

## COMPARISON OF SELECTED HABITATS BY AVERAGE VALUES

In the preceding sections we have drawn comparisons between the constants for the juices of the same species in the cases in which we were able to secure determinations from individuals growing in different habitats. It is now desirable to compare the selected habitats among themselves by means of averages. This may be most conveniently done by reference to tables, II to VI. In each of these tables the mean value of the osmotic concentration for a particular habitat is given in the second column for each of the growth forms considered. A comparison of these values with those for the four other habitats, entered at the tops of the remaining columns, and with those for the region as a whole, has been made in two different ways.

First, the differences between the averages appear in the body of the table. These are the entries with plus or minus signs. The subtractions are so made that the sign shows at once whether the corresponding value in the second column is larger (positive sign) or smaller (negative sign) than the value with which it is compared. Thus, in table II, the difference  $+ 0.13$ , for trees and shrubs of the rocky slopes, denotes that this amount must be added to the average concentration obtained from this growth form in this habitat, in order to obtain the corresponding concentration for the trees and shrubs of the canyons. In other words, when the osmotic value for trees and shrubs of the canyons is compared to that for the same growth forms of the rocky slopes, the canyon value is 0.13 atm. greater than the

other. Tables II to VI are all to be read in a similar way, as far as the quantities expressed with signs are concerned.

This method of comparison by differences, involving as it does only absolute values, has the disadvantage that the absolute differences, unless accompanied by the actual values of the two variables are not comparable among themselves. For this reason, the comparisons just described are also expressed in these tables, in the form of ratios, which are given beneath the corresponding differences, in blackface type. By this method of comparison any value expresses the ratio of the corresponding osmotic concentration given in the second column, to the concentration in question. Thus, from table II it appears that the concentration for trees and shrubs of the canyons is 1.01 as great as that for these same growth forms of the rocky slopes. The blackface values of tables II to VI are all to be read in a similar way.

From table II it appears at once that the plants of the canyons give, on an average, a lower osmotic value than do those of any other habitat except the arroyo. The only exception to this rule is found in the case of trees and shrubs from the rocky slopes and this is quite possibly attributable to the small number of constants available. The absolute difference is trivial in amount and the ratio is sensibly unity. For each of the four growth forms the osmotic value for the plants from the canyons is numerically, though not always significantly, higher than that of the plants collected in the arroyo.

The rocky slopes have a vegetation intermediate between that of the other habitats considered. The juices of these forms have concentrations higher than those of the forms found in the arroyos and canyons (with the exception noted above) but lower than those of the plants of the bajada and salt spots. The ratios show that the osmotic concentration of the tissue fluids of the plants of the rocky slopes is only about half as great as that of the species examined from the salt spots, but that it is about thirty per cent. higher than that of plants from the arroyo.

Table IV shows that the plants of the bajada have higher osmotic values than those of any other habitat except the salt spots. There is not a single exception to this rule in any of the four growth forms. The ratios show that the differences are relatively large.

The plants of the arroyo yield, without a single exception, (table V) saps giving a slighter, though perhaps not always a significantly smaller, depression of the freezing point, than those from any other of the selected environments. The ratios range from 0.37 to practically 1.00 in the narrowest subdivisions of the materials. Taking the plants as a whole, without regard to vegetation form, it is clear that those from the arroyo give sap only from 37 to 80 per cent. as concentrated as that from species occurring in the other habitats.

Finally, the species growing in the salt spots have, as shown in table VI, higher concentration than those of any other habitat. For the final units

TABLE II

*Comparisons of osmotic concentrations of expressed sap from plants growing in the canyons, with corresponding concentrations from plants of other habitats*

GROWTH FORM	CANYONS	CANYONS COMPARED TO OTHER HABITATS				
		Rocky Slopes	Bajada Slopes	Arroyo	Salt-Spots	All Habitats Together
Trees and shrubs.....	22.43	+0.13	-12.36	+4.72	-25.52	-5.67
	1.00	1.01	0.65	1.27	0.47	0.80
Dwarf and half-shrubs...	19.42	-2.45	-3.92	+2.84	-14.78	-2.03
	1.00	0.89	0.83	1.17	0.57	0.91
Perennial herbs.....	13.88	-3.14	-5.80	+0.30	.....	-2.47
	1.00	0.82	0.71	1.02	.....	0.85
Winter annuals.....	12.93	-2.33	-8.20	+0.01	-10.64	-1.80
	1.00	0.85	0.61	1.00	0.55	0.88
All species.....	17.34	-1.31	-9.07	+3.44	-19.75	-1.93
	1.00	0.93	0.66	1.25	0.47	0.90

TABLE III

*Comparisons of osmotic concentrations from plants growing on rocky slopes with those from plants of other habitats*

GROWTH FORM	ROCKY SLOPES	ROCKY SLOPES COMPARED TO OTHER HABITATS				
		Canyons	Bajada Slopes	Arroyo	Salt-Spots	All Habitats Together
Trees and shrubs.....	22.30	-0.13	-12.49	+4.59	-25.65	-5.80
	1.00	0.99	0.64	1.26	0.47	0.79
Dwarf and half-shrubs...	21.87	+2.45	-1.47	+5.29	-12.33	+0.42
	1.00	1.13	0.94	1.32	0.64	1.02
Perennial herbs.....	17.02	+3.14	-2.66	+3.44	.....	+0.67
	1.00	1.23	0.86	1.25	.....	1.04
Winter annuals.....	15.26	+2.33	-5.87	+2.34	-8.31	+0.53
	1.00	1.18	0.72	1.18	0.65	1.04
All species.....	18.65	+1.32	-7.76	+4.75	-18.44	-0.62
	1.00	1.08	0.71	1.34	0.50	0.97

TABLE IV

*Comparisons of osmotic concentrations from plants growing on bajada slopes with those from plants of other habitats*

GROWTH FORM	BAJADA SLOPES	BAJADA SLOPES COMPARED TO OTHER HABITATS				
		Canyons	Rocky Slopes	Arroyo	Salt-Spots	All Habitats Together
Trees and shrubs.....	34.79	+12.36	+12.49	+17.08	-13.16	+6.69
	1.00	1.55	1.56	1.96	0.73	1.24
Dwarf and half-shrubs...	23.34	+3.92	+1.47	+6.76	-10.86	+1.89
	1.00	1.20	1.07	1.41	0.68	1.09
Perennial herbs.....	19.68	+5.80	+2.66	+6.10	.....	+3.33
	1.00	1.42	1.16	1.45	.....	1.20
Winter annuals.....	21.13	+8.20	+5.87	+8.21	-2.41	+6.40
	1.00	1.63	1.38	1.64	0.90	1.43
All species.....	26.41	+9.04	+7.76	+12.51	-10.68	+7.14
	1.00	1.52	1.42	1.90	0.71	1.37

TABLE V

*Comparisons of osmotic concentrations from plants growing in the arroyo with those from plants of other habitats*

GROWTH FORM	ARROYO	ARROYO COMPARED TO OTHER HABITATS				
		Canyons	Rocky Slopes	Bajada Slopes	Salt-Spots	All Habitats Together
Trees and shrubs.....	17.71	-4.72	-4.59	-17.08	-30.24	-10.39
	1.00	0.79	0.79	0.51	0.37	0.63
Dwarf and half-shrubs...	16.58	-2.84	-5.29	-6.76	-17.62	-4.87
	1.00	0.85	0.76	0.71	0.48	0.77
Perennial herbs.....	13.58	-0.30	-3.44	-6.10	.....	-2.77
	1.00	0.98	0.80	0.69	.....	0.83
Winter annuals.....	12.92	-0.01	-2.34	-8.21	-10.65	-1.81
	1.00	1.00	0.85	0.61	0.55	0.88
All species.....	13.90	-3.44	-4.75	-12.51	-23.19	-5.37
	1.00	0.80	0.74	0.53	0.37	0.72

TABLE VI

*Comparisons of osmotic concentrations from plants growing in salt-spots with those from plants of other habitats*

GROWTH FORM	SALT-SPOTS	SALT-SPOTS COMPARED TO OTHER HABITATS				
		Canyons	Rocky Slopes	Bajada Slopes	Arroyo	All Habitats Together
Trees and shrubs.....	47.95	+25.52	+25.65	+13.16	+30.24	+19.85
	1.00	2.14	2.15	1.38	2.71	1.71
Dwarf and half-shrubs...	34.20	+14.78	+12.33	+10.86	+17.62	+12.75
	1.00	1.76	1.56	1.47	2.06	1.60
Perennial herbs.....	.....	.....	.....	.....	.....	.....
Winter annuals.....	23.57	+10.64	+8.31	+2.44	+10.65	+8.84
	1.00	1.82	1.54	1.12	1.82	1.60
All species.....	37.09	+19.76	+18.44	+10.68	+23.19	+17.82
	1.00	2.14	1.99	1.40	2.67	1.92

TABLE VII

*Comparisons of averages of osmotic concentrations from the different habitats and from all habitats together; the value for the arroyo is taken as unity in each series*

GROWTH FORM	ARROYO	CANYONS	ROCKY SLOPES	BAJADA SLOPES	SALT-SPOTS	ALL HABITATS
Trees and shrubs .....	1.00	1.27	1.26	1.96	2.71	1.59
Dwarf and half-shrubs .....	1.00	1.17	1.32	1.41	2.06	1.69
Perennial herbs .....	1.00	1.02	1.25	1.45	1.82	1.20
Winter annuals.....	1.00	1.00	1.18	1.64	.....	1.14
All species.....	1.00	1.25	1.34	1.90	2.67	1.39

of classification the ratios show that the osmotic value of the sap of these species is from 1.12 to 2.71 times as great as are the values from any other habitat. Unfortunately our series of determinations for the salt spots is too small to make the comparisons of great numerical value.

In the preceding tables the relationships of the values found in each habitat, to those of every other locality, have been shown. It will now be useful to express all of the values in terms of the corresponding constants for the arroyo considered as unity, which is done in table VII. This arrangement probably gives the best general conception of the range of differences in the several habitats considered. From table VII it appears, for example, that the osmotic value for trees and shrubs of the canyons is 1.27 times as great as that for the same growth forms of the arroyo, etc.

It should finally be noted that the order of magnitude of the osmotic values for the various growth forms considered is much the same for all five habitats. For the arroyo and for the canyons this order is identical; beginning with the lowest value, the series for these three habitats is: (1) Winter annuals, (2) perennial herbs, (3) dwarf shrubs and (4) trees and shrubs. For the salt spots the order is the same as far as shown, but no determinations are available for perennial herbs. For the rocky slopes the order is the same but the values for dwarf shrubs and for trees and shrubs are practically equal. For the bajada slopes the order is again the same except that the winter annuals yield a sap which is on the average more (but probably not significantly more) concentrated than that of the perennial herbs. Thus, if the probable errors of means based upon small numbers of observations be taken into account, there is probably not a single valid exception to the rule that osmotic concentration increases from winter annuals to perennial herbs, from perennial herbs to dwarf shrubs and half-shrubs, and from these to the more arborescent ligneous perennials.

### CONCLUDING REMARKS

In the foregoing pages we have shown that, in the plant species of the Arizona deserts studied by us, differences in the osmotic concentration of the sap, as determined by the depression of the freezing point of the expressed juices, are associated with differences in edaphic conditions. The evidences upon which this conclusion is based are given in detail in the body of the paper and are summarized in the immediately preceding section. They need not, therefore, be restated here.

The detailed comparison of the flora of the Tucson desert region as a whole with that of other desert, mesophytic and hygrophytic areas is yet to be drawn. In a preliminary comparison we have shown elsewhere that, with respect to the osmotic concentrations of their juices, the Tucson and the Cold Spring Harbor floras are conspicuously differentiated.

These results, supplemented by others, as yet unpublished, (for Long Island habitats, for Jamaican mangrove swamps, coastal deserts, and montane rain-forests, and for a series of habitats in subtropical Florida) lead to the conclusion that the study of the physico-chemical properties of the expressed plant sap must become an indispensable part of thorough-going ecological and phytogeographical investigation, just as the description of the peculiarities of the external morphology and internal structure of the species of a flora has long been recognized to be.

Without attempting to minimize in any way the importance of the macroscopic and microscopic morphological aspect of ecological work, we may point out that these deal principally with skeletal structures. The cell sap on the other hand has an intimate relation to the physiological activities of the living substance itself. It is, indeed, simultaneously the product and the environment of the somatic and germinal protoplasms.

The question to which most biologists would like to pass at once is that of the causes of the differences demonstrated. It is perhaps obvious that the differentiation of the plants of the different habitats may be in part due to innate differences in the osmotic concentration of the sap of the various species peculiar to the several habitats, and in part to a direct influence of the environment upon the sap properties of the individual plants. The discussion of this subject lies quite outside the scope of the present paper. We will merely warn those who feel inclined to settle it at once on theoretical grounds that it is a problem of great complexity, which may be solved only by the critical study of many kinds of data. Towards such a system of data the coefficients set forth in this memoir are one contribution.

The actual data here presented seem to us the most valuable feature of this study. They have their bearing upon a number of problems and will be utilized in comparisons to be made later. They are placed on record with the conviction that it is upon the comparative study of physical, chemical and biometric coefficients, determined with ever increasing accuracy, that the advancement of plant physiology and ecology primarily depends.

Finally we take pleasure in stating that the identification of our plants is due almost exclusively to Professor J. J. Thornber, of the University of Arizona. We consider ourselves particularly fortunate to have been able to secure the cooperation of a botanist with such intimate knowledge of the flora of the Southwest, in this essential feature of the work.

To express adequately our obligation to Dr. MacDougal and the members of the Staff of the Desert Laboratory would require more than a formal statement. Acknowledgment is made with such pleasant recollections of time spent in the deserts as only those who have themselves worked there will fully appreciate.

STATION FOR EXPERIMENTAL EVOLUTION  
COLD SPRING HARBOR, L. I.

## LITERATURE CITED

Numbers in brackets throughout the preceding pages refer to the year of publication and to the corresponding numbers that follow authors' names in this list. Where more than one reference to the same year occurs under a given name, these are serially numbered in italics, within the brackets.

- CANNON, W. A. [1911] The root habits of desert plants. Carnegie Inst. Wash. Pub. 131. Washington, 1911.
- [1913] Some relations between salt plants and salt spots. Dudley Memorial Volume, pp. 122-129. Leland Stanford Junior Univ., 1913.
- CAVARA, F. Risultati di una serie di ricerche crioscopiche sui vegetali. Cont. Biol. Veg. R. Ist. Bot. Palermo 4: 41-81. 1905.
- COVILLE, F. V., AND D. T. MACDOUGAL. Desert Botanical Laboratory of the Carnegie Institution. Carnegie Inst. Wash. Pub. 6. Washington, 1903.
- DIXON, H. H. Transpiration and the ascent of sap in plants. London, 1914. (Reference is made to this work as a summary of the several researches published by Dixon and Atkins.)
- DRABBLE, E., AND H. DRABBLE. The relation between the osmotic strength of cell sap in plants and their physical environment. Biochem. Jour. 2: 117-122. 1907.
- FITTING, H. Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitsch. Bot. 3: 209-275. 1911. (For critical discussion see Livingston [1911].)
- GORTNER, R. A., AND J. ARTHUR HARRIS. Notes on the technique of the determination of the depression of the freezing point. Plant World 17: 43-53. 1914.
- GORTNER, R. A., J. V. LAWRENCE AND J. ARTHUR HARRIS. The extraction of sap from plant tissue by pressure. Biochem. Bull. *In press*.
- HALKET, A. C. On various methods for determining osmotic pressures. New Phytologist 12: 164-176. 1913.
- HARRIS, J. ARTHUR. [1916, 1] The variable desert. Sci. Monthly. *In press*.
- [1916, 2] An extension to 5.99° of tables to determine the osmotic pressure of expressed vegetable saps from the depression of the freezing point. Amer. Jour. Bot. 2: 418-419. 1915.
- HARRIS, J. ARTHUR, AND R. A. GORTNER. Note on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point, with a table for the values of P for  $\Delta = .001$  to  $\Delta = 2.999$ . Amer. Jour. Bot. 1: 75-79. 1914.
- HARRIS, J. ARTHUR, J. V. LAWRENCE AND R. A. GORTNER. On the osmotic pressure of the juices of desert plants. Science N. S. 41: 656-658. 1915.
- LIVINGSTON, B. E. [1906] The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Wash. Pub. 50. Washington, 1906.
- [1911] The relation of the osmotic pressure of the cell sap in plants to arid habitats. Plant World 14: 153-164. 1911.
- LOYD, F. E. The artificial induction of leaf formation in the ocotillo. Torreya 5: 175-179. 1905.
- LOVEJOY, B. Gaertneria deltoidea Torr. Kans. Univ. Sci. Bull. 7: 277-288. 1913.
- MACDOUGAL, D. T. Botanical features of North American deserts. Carnegie Inst. Wash. Pub. 99. Washington, 1908.
- MACDOUGAL, D. T., AND W. A. CANNON. The condition of parasitism in plants. Carnegie Inst. Wash. Pub. 129. Washington, 1910.
- MARIE, C. H., AND C. L. GATIN. Determination cryoscopiques effectuées sur des sucres végétaux. Compt. rend. Assoc. Fr. Avanc. Sci. 40: 492-494. 1912.
- OHLWEILER, W. W. The relation between the density of cell saps and the freezing point of leaves. Ann. Rept. Missouri Bot. Gard. 23: 101-131. 1912.
- SREEVE, F. [1913] A guide to the salient features of the vicinity of Tucson, Arizona. Tucson, 1913.
- [1915] The vegetation of a desert mountain range as conditioned by climatic factors. Carnegie Inst. Wash. Pub. 217. Washington, 1915.
- SPALDING, V. M. [1904] The creosote bush (*Covillea tridentata*) in its relation to water supply. Bot. Gaz. 38: 122-138. 1904.
- [1906] Absorption of water by leaves. Bot. Gaz. 41: 262-282. 1906.
- [1909] Distribution and movements of desert plants. Carnegie Inst. Wash. Pub. 113. Washington, 1909.
- WOOTON, E. O., AND P. C. STANDLEY. Flora of New Mexico. U. S. National Herb. Cent. 19. 1915.



## FURTHER STUDIES ON FOLIAR TRANSPIRING POWER IN PLANTS

A. L. BAKKE AND B. E. LIVINGSTON

### ABSTRACT<sup>1</sup>

This paper presents the results of a large number of determinations of the transpiring power of leaves of *Xanthium canadense* Mill. and *Helianthus annuus* L., these being made on leaves of various ages and at various times of day and night. The Livingston method, employing slips of cobalt-chloride paper applied to the leaves, was used. A single plant of each species was studied, growing in the open at Tucson, Arizona.

All of the series of tests agree in showing a daily march in the average foliar transpiring power of the plant as a whole, from low night values to high day values and the reverse, as has already been shown for other plants.

The maximum value of the average index of foliar transpiring power occurred about the tenth hour of the day, in July and August. The actual values of these average maxima are shown to be 0.92, 0.96 and 0.81 for the three series employing *Xanthium*, and 0.86, 0.82, 0.78 and 0.85 for the four series employing *Helianthus*. Thus these two plants appear to have about the same diurnal maximum of foliar transpiring power, and may be considered as possessing similar degrees of foliar xerophitism.

The minima of average foliar transpiring power are shown to occur about the 18th hour of the day, or else about the 22nd or 23rd hour. The evidence as to the exact time of this occurrence is not satisfactorily complete. The actual average values obtained are 0.15 and 0.23 for *Xanthium*, and 0.22, 0.25 and 0.19 for *Helianthus*, from which it appears that these two plants are nearly alike in this respect also.

The ratio of average maximum to average minimum is shown as 3.5, 3.7, 3.1 and 4.5, respectively, for one series with *Xanthium* and three series with *Helianthus*. Another series with *Xanthium* shows a much higher ratio (6.4), so that this matter is left in doubt. It is probably safe to suppose that the diurnal transpiring power of these plants was about 3 to 5 times as great as was the nocturnal transpiring power.

Some evidence is furnished showing a secondary minimum following the day maximum in *Xanthium*, somewhat similar to such secondary minima as previously observed.

<sup>1</sup> The manuscript of this paper was received May 1, 1916. This abstract was preprinted, without change, from these types, and was issued as Physiological Researches Preliminary Abstracts, vol. 2, no. 2, July, 1916.

The very rapid increase in foliar transpiring power accompanying the occurrence of sunrise was investigated by observations taken just before and just after sunrise. This increase, within a single hour, amounts to as much as 240 per cent. in the case of the average value for *Xanthium*; it is not nearly as great for *Helianthus*, being only about 90 per cent. Whether this represents a physiological difference between these two plants is left as an unanswered question.

It appears generally clear that the oldest leaves on a shoot always have a low daily range of transpiring power, which seems to be due to relatively low maximum values, which are shown by these older leaves. This age relation is to be taken as only tentative. The various leaves of the plant all show a daily fluctuation in transpiring power as here measured, but there is frequently a marked lack of synchrony among them, especially as to the time of occurrence of the day maximum.

## INTRODUCTION

From work already reported<sup>2</sup> it appears that leaves of different position on the stem may differ markedly in transpiring power, as this is measured by means of the method of standardized hygrometric paper. Furthermore, from Livingston's studies<sup>3</sup> and from those just cited, it is clear that the transpiring power of any single leaf generally varies greatly throughout the day, having high values at some hours and low values at others. The present studies were planned to bring forth more information in these connections.

The work was begun in the summer of 1913 and was continued the following summer, at the Desert Laboratory of the Carnegie Institution of Washington, at Tucson, Arizona. We wish here to express our thanks to the Director of that Laboratory, Dr. D. T. MacDougal, for the facilities that he very kindly placed at our disposal.

The methods employed in 1914 were the same as those used during the previous summer, with the exception that a new apparatus for the standard evaporating surface was devised by Bakke and was used in the later work (see fig. 1.) The improvement consists in alterations that make it possible for the apparatus to be carried about in the pocket without danger of spilling the water. The essentials have not been changed. The new water-container<sup>4</sup> is made of aluminium throughout, being turned from a solid block

<sup>2</sup> Bakke, A. L., Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. *Jour. Ecol.* 2: 145-173. 1914.

<sup>3</sup> Livingston, B. E., The resistance offered by leaves to transpirational water-loss. *Plant World* 16: 1-35. 1913.

<sup>4</sup> For the construction of the apparatus I am indebted to Prof. W. H. Meeker, head of the Mechanical Engineering Department of this College, through whom I was enabled to obtain the assistance of Prof. John Hug, in making the detailed drawings, and of Mr. A. F. Nickels, who turned out the first instrument. I have since succeeded in having these instruments manufactured of brass, nickel-plated, by Wm. Gaertner & Co., of Chicago.—A. L. Bakke.

of metal. A thin aluminium disk with threads on its outer margin screws into the cylindrical reservoir to form the top. The top is 5 cm. in diameter and the reservoir is a cylinder 4 cm. high. Two openings are cut in the disk, about 1 cm. long and 1 mm. wide, the two being parallel and 2.5 cm. apart, equally distant from the center of the disk. A strip of blotting paper

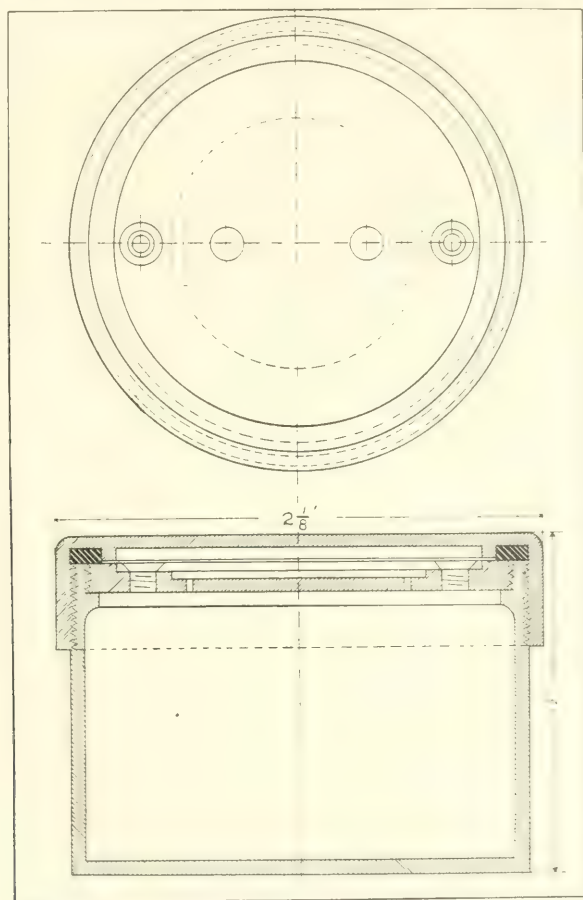


FIG. 1. Cross-sectional diagrams of new apparatus for standard evaporating surface. The heavily shaded portions represent the rubber gasket in the cap. Dimensions are in inches.

about 1 cm. wide is passed up through one of these openings and down through the other, the portion between the openings resting flat upon the upper surface of the plate. The two ends dip into water in the reservoir below. The disk has a slightly elevated margin, of a height equal to the thickness of the blotting paper, which allows a second disk to be placed over the first, so as

to lie against the horizontal part of the paper, but no pressure is thus applied to the paper. The top plate is similar to the one heretofore used, with two circular openings 8 mm. in diameter, so placed that both openings lie over the horizontal part of the paper strip. This second plate is set into the first and attached by two screws. It is thus removable, to allow the blotting paper strip to be replaced. An overlapping cap screws on to the basal portion, with a rubber gasket around the margin of the plane surface. When the instrument is closed the pressure of the air inclosed between the upper plate and the cap effectually prevents the escape of water, even though the instrument is inverted. The glass plate is the same as has been used heretofore, and is to be carried separately. The cap is of course removed and this glass plate is applied in its stead when the apparatus is in use. The paper used for impregnation with cobalt chloride was J. H. Munktel's "Unrivalled Swedish" filter paper, No. 00, the same as was advocated in Bakke's earlier paper, already cited, and the clips were the same as have been previously used.

The purpose of this study was to determine as well as possible the index of transpiring power for the whole plant, from measurements made on all the leaves instead of from those derived from representative ones only. At the same time it was planned to obtain a better insight than has heretofore been possible, into the differences in this index, between different leaves of the same plant, and into the variations of the index value as each leaf became older. A plant of *Xanthium canadense* Mill, growing in an arroyo near the office building of the Desert Laboratory, and one of *Helianthus annuus* L., growing in an abandoned irrigation ditch not far away, were chosen for this detailed study. The *Xanthium* plant was 18 cm. high and had five leaves on the main stem when the work was begun, and short secondary stems were present. There were altogether 10 leaves, whose dimensions varied from 4 x 4 cm. to 9 x 9 cm. The *Helianthus* plant was 34 cm. high and had 16 leaves at the start; the dimensions of the basal leaves were about 5 x 8 cm. and those higher on the stem were about 8 x 11 cm. For easy identification the leaves were numbered and tagged, from the base of the plant upward, so that larger numbers represent younger leaves. The very youngest leaves were not considered. In the case of *Xanthium* none were used smaller than 4 x 4 cm., and in the case of *Helianthus* the smallest leaves used were 3 x 6 cm. In the following paragraphs the experimental data for *Xanthium* are given first, followed by those for *Helianthus*.

## EXPERIMENTATION ON XANTHIUM

A series of tests (I) on the *Xanthium* plant was begun on July 20, at hour 8, and was continued, at 2-hour intervals, until hour 16, when rain occurred. Readings were taken on the different leaves in the order of their serial num-

bers, for each time of observation. It took nearly an hour to obtain all the readings, so that but little more than an hour elapsed between the end of one set of tests and the beginning of the next.

A second and similar series of observations (II) began at hour 6 of August 21 and continued till the same hour of the following day. These tests were made at irregular intervals, the observations being planned with the idea of bringing out the main features of the daily march of transpiring power.

The data obtained from these two series are brought together in table I. After hour 8 in series II, leaf 1 was injured, so that no more observations were obtained therefrom. In the lowest line of the table are given the averages of the indices for all leaves, for the corresponding hours, these

TABLE I

*Indices of transpiring power for the entire leaf surface of each of ten different leaves of a Xanthium plant, at various hours of the day and night, including two series of observations (July 20 and July 21-22). The leaves are serially numbered in the order of their age, beginning with the oldest. Maxima are in black-face type and minima in italics.*

LEAF NUMBER	SERIES I, JULY 20					SERIES II, JULY 21-22							RATIO OF MAX. TO MIN.
	Hr. 8	Hr. 10	Hr. 12	Hr. 14	Hr. 16	July 21		July 22					
						Hr. 18	Hr. 22	Hr. 4	Hr. 6	Hr. 10	Hr. 14	Hr. 18	
1	0.85	0.99	<b>1.09</b>	0.98	0.89	0.20	0.19	0.21	0.55				
2	0.77	0.97	<b>1.04</b>	0.85	0.92	0.36	0.18	0.21	0.61	<b>1.02</b>	0.56	0.42	5.7
3	0.80	0.84	0.87	0.93	<b>0.97</b>	0.32	0.17	0.20	0.64	<b>0.89</b>	0.49	0.41	5.2
4	0.88	0.88	0.85	0.89	<b>0.94</b>	0.72	0.16	0.20	0.56	<b>1.05</b>	0.46	0.17	6.6
5	0.89	<b>1.09</b>	0.76	0.79	0.90	0.07	0.15	0.21	0.68	<b>0.92</b>	0.62	0.08	13.1
6	0.98	<b>1.06</b>	0.89	0.86	1.02	0.10	0.16	0.28	0.89	<b>1.01</b>	0.55	0.15	10.1
7	0.75	<b>1.00</b>	0.82	0.74	0.88	0.06	0.10	0.25	0.72	<b>0.97</b>	0.39	0.16	16.2
8	0.74	<b>0.97</b>	0.74	0.53	0.94	0.10	0.10	0.25	0.88	<b>0.94</b>	0.48	0.14	9.4
9	0.97	<b>0.98</b>	0.77	0.57	0.76	0.08	0.11	0.24	1.13	0.97	0.40	0.14	12.1
10	<b>0.64</b>	0.47	0.35	0.31	0.33	0.06	0.14	0.21	0.74	<b>0.83</b>	0.40	0.12	13.8
Average	0.83	<b>0.92</b>	0.82	0.75	0.85	0.21	0.15	0.22	0.74	<b>0.96</b>	0.48	0.20	6.4

representing the foliar transpiring power of the entire plant. The maximum value for the period is indicated, for each leaf, by black-face type and the minimum for series II is shown by italics. This system of notation will be followed in later tables also.

The indices here given for the entire leaf were obtained, as heretofore, by averaging the index for the upper and that for the lower surface, at each observation. It will be remembered that the index for either leaf surface is derived by dividing the time required for color change of the hygrometric paper over the standard evaporating surface by the corresponding time required on the leaf surface in question.

A third series of observations (III) on the same plant was carried out on August 25 and 26, the data from which are given in table II. The main points brought out by each of these three series will now be presented in order.

#### SERIES I

The first series shows the day transpiring power of all leaves taken together (average of entire plant) to vary between 0.92 (hour 10) and 0.75 (hour 14), but the individual leaves varied far beyond these limits. The highest leaf index obtained in this series is 1.09 (leaf 1, hour 12, and leaf 5, hour 10). The lowest value is 0.31 (leaf 10, hour 14).

Considering the march of these values for the different leaves, it appears that leaves 5 to 9, inclusive, show maximum values at the tenth hour, the same hour as that at which occurred the maximum for the entire plant. Leaves 1 and 2 had their maxima later; for leaf 1 the maximum was apparently extended to include hours 12 and 14, while leaf 2 had its maximum at the twelfth hour. Leaves 3 and 4 show no maxima in the middle of the day; the index values for leaves 3 and 4 are low until the last 2 hours of the series, when they increase considerably. Leaf 10 is exceptional in that its index has its highest value at the eighth hour, followed by a general decrease to hour 14. It is to be noted, also, that all values of leaf 10 are exceptionally low. This is the youngest of the leaves dealt with. The daily maximum is thus shown, in this case, to be late for the two oldest leaves (hour 12) and early for the youngest one.

After passing the maximum in the middle part of the day the index value decreases (for all but leaves 3, 4 and 10) and then reaches a secondary minimum. This occurs at hour 14 for leaves 2, 6-10, and at hour 12 for leaf 5. The average value for the entire plant also attains a minimum at hour 14. In the case of leaf 1 the decrease continues to the end of the series, the maximum being late in this case, as has been mentioned. The minima for leaves 3 and 4, so far as they are indicated, occur at hours 10 and 12, respectively. In the case of leaf 10 this afternoon minimum is merely suggested, at hour 14.

The minimum just considered is followed, within the limits of this series, by an increase in the index value in all but leaf 1, though the general march of the index for leaves 3 and 4 is exceptional, as has been pointed out. In general, it appears from series I that transpiring power rises rapidly in the early forenoon, passes a maximum, and then falls to a temporary minimum after which it again rises. The shape of the average graph for series I is very similar to that presented for *Quamoclidion* in an earlier paper (Bakke [1914], p. 152). This temporary fall in transpiring power seems also to be similar to that described by Edith B. Shreve,<sup>5</sup> for *Parkinsonia*.

<sup>5</sup> Shreve, Edith B., The daily march of transpiration in a desert perennial. Carnegie Inst. Wash. Pub. 194. Washington, 1914.

## SERIES II

The second series with *Xanthium* was longer than the first, but many of the intervals between observations were also longer, so that this series does not bring out details for the hours 8 to 16 as well as does the earlier one. The graph for this series, for all of the leaves together, is here reproduced as figure 2. It is clear that this average index value is low throughout the night, rises to a maximum (0.96) at the tenth hour and then falls to the low night value, which is attained about hour 18. No indication of a temporary minimum in the day-time, followed by a secondary maximum—such as

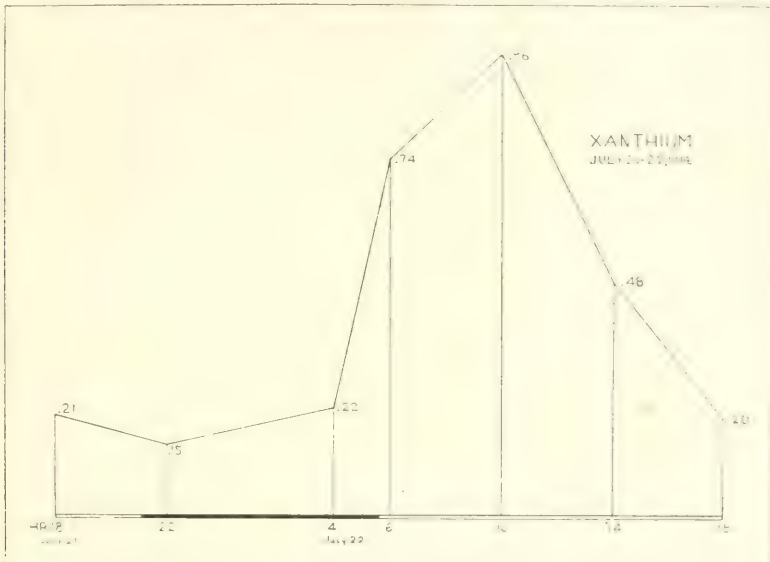


FIG. 2. Graph showing daily march of average foliar transpiring power of *Xanthium* plant, from hour 18 of July 21 to the same hour of the following day. Hours of darkness are indicated by broad, full line below. Data are from table I, series II.

appeared from series I—is here apparent, but this may possibly be due to the lack, in this case, of any observations between hours 14 and 18, which represent the period within which this secondary maximum might be expected.

The night values, which are not shown at all by series I, are all below 0.20 for hour 22. The minimum appears to be high for the oldest leaves and low for the youngest. All the individual leaf maxima occur at hour 10, with the average maximum—except that for leaf 9, which appears at hour 6. While this value for leaf 9 is very high (1.13), the maxima for the other leaves are much lower, varying from 0.83 to 1.05, without any apparent relation to the age of the leaves.

The most interesting feature of this series lies in the rapid fall here shown

in every case, after the maximum is passed. Excepting in the case of the youngest leaf (10), all of the values here given for hour 14 are markedly lower than those obtained for the same hour of the first series. As has been noted, there may have been secondary maxima about hour 16, but this series presents no evidence on that point. If such maxima actually occurred, they were probably much lower than the corresponding ones of series I, however. What may have produced this difference between the two series cannot, of course, be stated, but it may be mentioned that the period of the later series was without rain while that of the earlier one was terminated by a shower. The slower decrease in the index value for series I, after passing its maximum, may be related to lower atmospheric evaporating power and lower radiation intensity, and the high values recorded for hour 16 in the earlier series may possibly be related to the approach of rain. Much more detailed experimentation will be necessary, however, before such questions as this can be settled definitely. The diurnal march of transpiring power for the youngest leaf in series I is similar to that for the next to the youngest in series II.

Turning to the extremes recorded for the individual leaves in series II, these are set forth in table I, where maxima are again shown in black-face type and minima in italics. The table also gives the ratios between corresponding maxima and minima for this series. From these data it appears that the three oldest leaves showed maxima only 5 or 6 times their minima, while the rest showed from 9 to 16 times as great values at their maxima as at their minima. As has been noted, there seems to be no relation between leaf-age and the value of the maximum, but the three highest minima are those for the three oldest leaves. It is thus suggested that the oldest leaves fail to retard transpiration in the night quite as much as do the younger ones. It also appears that hours 10 to 16 show relatively low values for the youngest leaves (9 and 10), as if these values tended, throughout the day, to remain more nearly the same as the corresponding night values, while the older leaves increased their transpiring power markedly for the daylight period.

#### SERIES III

On August 25 and 26 a third series of observations was carried out on this *Xanthium* plant. The same leaves as were used in series I and II could not be employed, however, for flood water in the arroyo had injured the plant; of the original lot, only leaves 8 and 10 were left and these fell when the clip was applied. Healthy branches had developed from the axils of leaves 8 and 10, the first with seven leaves and the other with 6, of suitable size for these tests, and series III was carried out with these thirteen leaves. This series began with hour 18 of the first day and continued to include hour 22 of the next. Observations were taken always in the order of the serial

leaf-numbers. The hours of observation and the data obtained are set forth in table II. The ratio of the maximum to the minimum value is also given, for each leaf, in the last column. Since two branches are considered, the averages have been computed for each branch separately and they are so given in table II. The data for these two separate branches will be referred to as series IIIa (leaves 8-1 to 8-7) and series IIIb (leaves 10-1 to 10-6).

Considering the averages for the entire plant, low night values and high day values are shown for both branches. Both average maxima occur with

TABLE II

*Indices of transpiring power for the entire leaf surface of each of 13 different leaves of a Xanthium plant, at various hours of the day and night. The leaves were on two branches, those on one branch being numbered from 8-1 to 8-7; those on the other, from 10-1 to 10-6. In both cases the second number denotes the relative age of the leaf, the highest numbers referring to the youngest leaves. Maxima are in black-face type and minima in italics.*

LEAF NUMBER	AUGUST 25	AUGUST 26					RATIO OF MAX. TO MIN.
	Hour 18	Hour 6	Hour 10	Hour 14	Hour 18	Hour 22	
8-1	0.22	0.23	<b>0.40</b>	<i>0.20</i>	0.21	0.26	2.0
8-2	0.37	0.28	0.64	<b>0.71</b>	0.35	<i>0.23</i>	3.1
8-3	0.25	0.25	<b>0.86</b>	0.69	0.38	<i>0.23</i>	3.7
8-4	0.35	<i>0.29</i>	<b>0.92</b>	0.69	0.40	0.32	3.2
8-5	0.24	0.31	<b>0.98</b>	0.51	<i>0.23</i>	0.27	4.3
8-6	<i>0.18</i>	0.38	<b>0.81</b>	0.49	0.22	0.26	4.5
8-7	0.22	0.47	<b>0.84</b>	0.47	0.27	<i>0.20</i>	4.2
Average	0.26	0.32	<b>0.78</b>	0.54	0.29	<i>0.25</i>	3.1
10-1	<i>0.13</i>	0.20	<b>0.40</b>	0.18	0.20	0.18	3.1
10-2	0.24	0.35	<b>0.87</b>	0.47	0.21	<i>0.17</i>	5.1
10-3	0.23	0.25	<b>0.94</b>	0.62	<i>0.21</i>	0.31	4.5
10-4	<i>0.13</i>	0.32	<b>0.97</b>	0.67	0.20	0.31	7.5
10-5	<i>0.18</i>	0.27	<b>1.00</b>	0.59	0.31	0.22	5.6
10-6	<i>0.25</i>	0.28	<b>0.94</b>	0.58	0.32	0.26	3.8
Average	<i>0.19</i>	0.28	<b>0.85</b>	0.52	0.24	0.24	4.5

hour 10, and the minimum occurs with hour 22 for the first branch and with hour 18 for the second. For the second branch the minimum is lower and the maximum higher than for the first, and the (ratios) are, respectively, 3.1 and 4.5. Neither of these branches exhibits any evidence of a secondary minimum in the day, shown for series I, but such a minimum may have occurred between hours 10 and 14.

Considering the individual leaves, in every case but that of leaf 8-2 the maximum for the day is here shown as occurring with the 10th hour; in this single exception the maximum value occurred with the 14th hour. Of

course observations were too infrequent to show definitely just when the maximum was actually reached for any of these leaves. On the first branch, the maximum value is very low (0.40) for leaf 8-1, and rather low for leaf 8-2. The minimum is low for leaves 8-1, 8-6 and 8-7. Turning to the other branch, leaf 10-1 shows a very low maximum (0.40, the same as that of 8-1), and all the other leaves on this branch show maxima somewhat above the average. The magnitude of the leaf minima do not here appear to be related to the age of the leaves. Also, the times of occurrence of maximum and minimum do not appear to be related to leaf-age, for either branch. The oldest leaf (8-1, 10-1) exhibited a very low daily range of transpiring power in both cases. This range is seen to have been low, not because the minimum (night) value was high (as was suggested from series II), but because the maximum (day) value was low. These two oldest leaves were yellowing and somewhat flaccid. The younger leaves show a high range for the first branch and the youngest (10-6) shows a low one for the other branch.

#### SUMMARY OF RESULTS OF SERIES I-III, XANTHIUM

The relation of leaf-age to transpiring power, as brought out in these series with the *Xanthium* plant, may be summarized as follows:

Oldest leaves have low maxima for series IIIa and IIb, while youngest have low maxima for series I and II.

Oldest leaves have high minima for series II and low ones for series IIIa, while youngest have low minima for series II and IIIa. Series I presents no evidence in this regard.

Oldest leaves show late maxima in series I, while youngest have early maxima for series I and II.

Range of index value is relatively low for oldest leaves in series II, IIIa and IIb; it is relatively high for youngest leaves in series IIIa, but relatively low in series IIb. Series I presents no evidence regarding range.

From these statements it may be concluded that only one special relation between leaf-age and transpiring power is evident from these tests; namely, that a low daily range of transpiring power is clearly indicated for the oldest leaves in all three cases that bear upon this point. It appears that this question of the relation of transpiring power to age of leaf, or to leaf-position on the stem, is a very complicated one, this relation seeming to be altered with the branch, with the hour of the day, and perhaps with the climatic and soil conditions.

#### RELATION OF SUNRISE TO TRANSPIRING POWER IN XANTHIUM

A special set of observations made just before and just after sunrise, on the *Xanthium* plant, emphasizes the rapid rise in transpiring power that

occurs with sunrise. This short series was carried out on July 19, the day before the date of series I, and the plant was in practically the same condition as for that series. The first of these two sets of observations occurred between 4:15 and 4:45, before dawn, and the second occurred between 6:25 and 6:45, directly after sunrise. The results are presented in table III, which also gives, as examples, the actually observed times for color changes

TABLE III

*Data of foliar transpiring power for ten different leaves of a Xanthium plant, obtained during the 5th hour and also during the 6th hour of July 19, to show alteration in the index value accompanying sunrise, which occurred about 5:30.*

LEAF NUMBER	TIME OF OBSER- VATION	TIME OF COLOR CHANGE		INDEX OF TRANSPIRING POWER					
		Lower surface	Upper surface	Lower surface		Upper surface		Entire leaf	
				Index	Ratio	Index	Ratio	Index	Ratio
		<i>sec.</i>	<i>sec.</i>						
1	4:15	230	263	0.22	3.0	0.19	2.2	0.21	2.6
	6:25	65	105	0.67		0.42		0.55	
2	4:15	248	245	0.20	2.7	0.21	3.2	0.21	2.9
	6:25	80	65	0.55		0.67		0.61	
3	4:15	243	273	0.21	3.5	0.19	2.9	0.20	3.2
	6:25	60	80	0.73		0.55		0.64	
4	4:25	242	260	0.21	2.8	0.20	2.7	0.20	2.8
	6:30	75	82	0.58		0.53		0.56	
5	4:30	200	297	0.25	3.5	0.17	2.9	0.21	3.2
	6:30	50	90	0.88		0.49		0.68	
6	4:30	170	200	0.30	3.0	0.25	3.4	0.28	3.2
	6:40	48	51	0.91		0.86		0.89	
7	4:30	210	200	0.24	3.1	0.25	2.9	0.25	2.9
	6:40	59	62	0.74		0.72		0.72	
8	4:45	170	257	0.30	3.2	0.20	3.9	0.25	3.5
	6:45	45	56	0.97		0.78		0.88	
9	4:45	245	190	0.21	5.1	0.27	4.4	0.24	4.7
	6:45	41	37	1.07		1.18		1.13	
10	4:45	200	320	0.25	3.8	0.16	3.3	0.21	3.5
	6:45	46	84	0.95		0.52		0.74	
Average	Hr. 5			0.24	3.5	0.20	3.4	0.22	3.4
	Hr. 6			0.83		0.67		0.75	

of the hygrometric paper on the upper and on the lower leaf surfaces. The time period required for color change over the standard evaporating surface was 50.75 sec. for the earlier set of tests, and 43.8 sec. for the later one. This table also gives, in each case, the ratio for the entire leaf, of the index value just after sunrise to that just before.

From table III it appears that the increase in foliar transpiring power for the entire leaf, occurring with sunrise, was somewhat greater for the youngest

three leaves (8, 9 and 10) than for any of the others. The smallest increase is shown for the oldest leaf (1). Leaves 2-7 are to be regarded as about alike in this respect. But it is to be remembered that these pairs of observations do not all refer to the same time interval; the first observation on the youngest leaves was made half an hour earlier than was the first on the oldest ones.

Comparing the change recorded for the upper with that for the lower leaf-surface, it is seen that the ratios of the averages are about alike (3.5 for the lower and 3.4 for the upper). It is interesting to note, however, that this relation does not hold uniformly for the individual leaves. In over two-thirds of the cases the lower surface shows a greater change than does the upper, but in three cases (leaves 2, 6 and 8) the alteration is greater for the upper leaf surface. These differences do not appear to be related to the age of the leaves.

The average index value for the entire plant is 3.4 times as great for the sixth hour as for the fifth. This very rapid change seems almost certainly to be due mainly to the opening of the stomata.

## EXPERIMENTATION ON HELIANTHUS

Three series of observations were made on the *Helianthus* plant, to study the daily march of foliar transpiring power for the various leaves. The first (I) was carried out on July 28, when the plant had 16 leaves large enough for the purpose. The second series (II) was carried out on July 31 and August 1, 18 leaves being employed. The third series (III) was carried out on August 24 and 25, using leaves numbered from 6 to 32. The data for these three series will now be presented, followed by those for a special series dealing with the relation of sunrise to the magnitude of foliar transpiring power in *Helianthus*.

### SERIES I

This series began with hour 5 of July 28 and continued till hour 17 of the same day, when rain brought it to a close. Leaves numbered from 1 to 16 were employed, no. 1 being the oldest and no. 16 the youngest. Observations were not obtained in the same order at the different times, but the time of observation was recorded for each leaf, to the nearest 5-minute interval. Three different observations were obtained on each leaf, one between 5:15 and 6:30, one between 9:20 and 9:55, and the third between 13:15 and 13:55. The index values (for the entire leaf in each case) are shown in table IV.

Sunrise occurred on this day at about 5:40, and those values obtained at or before this time are indicated by italics in the third column of table IV. It will be observed that all these values are very small (from 0.14 to 0.27)

and that the values obtained after sunrise are much larger (from 0.40 to 0.63). From what has been shown for *Xanthium* and from data to be given below, it may be supposed that those leaves not observed before sunrise would also have shown these very low values if they had been observed at the proper time. The second set of observations (9:20-9:55) show much higher values than were obtained at the first. As far as this series goes, it shows the maximum values for the day as occurring at the time of the second observation with the exception of leaves 1 and 2, for which the index values were still larger at 13:15.

TABLE IV

*Indices of foliar transpiring power (entire leaf) for each of 16 leaves of a Helianthus plant, at three different hours of the day, July 28. Maxima are in black-face type and minima in italics.*

LEAF NUMBER	FIRST OBSERVATION		SECOND OBSERVATION		THIRD OBSERVATION	
	Hour	Index	Hour	Index	Hour	Index
1	5:15	0.14	9:20	0.56	13:15	<b>0.72</b>
2	5:30	0.16	9:20	0.63	13:15	<b>0.76</b>
3	6:10	0.43	9:20	<b>0.72</b>	13:30	0.56
4	6:05	0.40	9:20	<b>0.65</b>	13:45	0.47
5	5:55	0.43	9:25	<b>0.79</b>	13:45	0.59
6	5:15	0.15	9:25	<b>1.01</b>	13:15	0.72
7	5:30	0.20	9:25	<b>0.80</b>	13:30	0.79
8	6:05	0.49	9:35	<b>0.98</b>	13:45	0.78
9	6:15	0.50	9:45	<b>0.98</b>	13:40	0.76
10	5:15	0.21	9:45	<b>0.95</b>	13:20	0.92
11	5:40	0.16	9:50	<b>0.95</b>	13:45	0.59
12	5:50	0.43	9:50	<b>0.95</b>	13:40	0.84
13	5:40	0.22	9:50	<b>0.98</b>	13:55	0.84
14	6:20	0.63	9:55	<b>0.95</b>	13:20	0.82
15	5:20	0.27	9:55	<b>0.87</b>	13:55	0.83
16	6:20	0.60	9:55	<b>0.93</b>	13:55	0.82
Average	.....	0.34	.....	0.86	.....	0.74

The general conclusion from this series is that the transpiring power of these leaves (just as in the case of *Xanthium*) rises from a minimum occurring some time in the night to a maximum in the day, about hour 10, but the hour of this maximum is not well shown here. The oldest two leaves were apparently later in attaining their maximum than were any of the others. It is also suggested that the older leaves (say nos. 1 to 4) show actually lower index values for the second observation than do the younger ones, but since these younger leaves were observed somewhat earlier than were the older ones this point is not clearly established.

## SERIES II

The second series of observations on the *Helianthus* plant was carried out between hour 18 of July 31 and the same hour of the following day. The same leaves were used as in series I for this plant, with the addition of leaves 17 and 18, these being the youngest of the present series. Readings were taken in the order of the leaf numbers, being begun at the time indicated in table V, which presents the data obtained. Rain prevented all the leaves being observed at hour 18 of the second day.

The daily march of the average foliar transpiring power for all the leaves, as shown by the numbers in the last line of table V, is set forth graphically

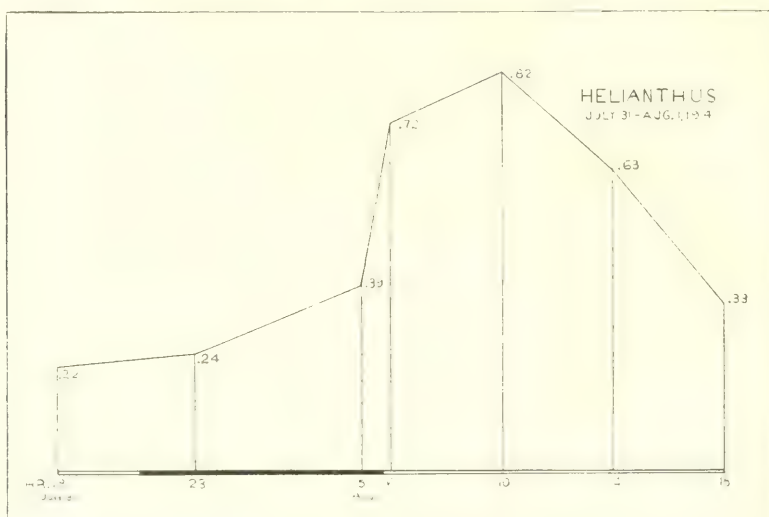


Fig. 3. Graph showing daily march of average foliar transpiring power of *Helianthus* plant, from hour 18 of July 31 to the same hour of the following day. Hours of darkness are indicated by broad, full line below. Data are from table V.

in figure 3, which is constructed in the same general way as the graph of figure 2. This average index is seen to increase rapidly in the early morning, attaining its maximum with hour 10, after which it decreases to hour 18. In general form this graph closely resembles the corresponding one for *Xanthium* shown in figure 2.

For the older leaves (1-9) the lowest value occurred at hour 18, while five of the younger leaves—but not the youngest one (18)—showed their minima as occurring at hour 23. Since all of this younger group do not agree in this respect, this indication is not very clear; it may suggest that older leaves attain their minimum values earlier in the night than do younger ones. The maxima occur mainly at hour 10, leaves 4 and 5 being exceptions,

with apparent maxima at hour 6. Here, again, observations were not frequent enough to determine the time of occurrence of the maximum with satisfactory precision. The true maximum surely occurs between hour 6 and hour 14, for all leaves. Another point is suggested by this series; namely, that the midnight values (hour 23) are highest for the older leaves (2-8), while the day values (hours 10 and 14) are highest for the younger ones (7-18). The daily range of the index value, as shown by the ratios

TABLE V

*Indices of foliar transpiring power (entire leaf) for each of 18 leaves of a Helianthus plant, at seven different hours of the day, July 31-Aug. 1. Maxima are in black-face type and minima in italics.*

LEAF NUMBER	JULY 31		AUGUST 1					RATIO OF MAX. TO MIN.
	Hour 18	Hour 23	Hour 5	Hour 6	Hour 10	Hour 14	Hour 18	
1	<i>0.20</i>	0.23	0.41	0.57	<b>0.71</b>	0.41	<i>0.19</i>	3.5
2	<i>0.22</i>	0.29	0.38	0.63	<b>0.73</b>	0.45	.....	3.3
3	<i>0.25</i>	0.28	0.29	0.60	<b>0.72</b>	0.45	.....	2.9
4	<i>0.23</i>	0.28	0.34	<b>0.43</b>	0.29	0.35	.....	1.9
5	<i>0.19</i>	0.28	0.32	<b>0.58</b>	0.53	0.34	.....	3.1
6	<i>0.20</i>	0.28	0.45	0.71	<b>0.76</b>	0.46	.....	3.8
7	<i>0.16</i>	0.30	0.42	0.72	<b>0.81</b>	0.50	0.29	5.1
8	<i>0.18</i>	0.29	0.29	0.78	<b>0.88</b>	0.56	.....	4.9
9	<i>0.24</i>	<i>0.24</i>	0.30	0.65	<b>0.89</b>	0.51	.....	3.7
10	0.30	<i>0.22</i>	0.36	0.72	<b>0.93</b>	0.81	.....	4.2
11	0.21	<i>0.19</i>	0.44	0.69	<b>0.97</b>	0.73	0.33	5.1
12	<i>0.19</i>	0.24	0.36	0.79	<b>0.97</b>	0.79	.....	5.1
13	<i>0.17</i>	0.20	0.37	<b>0.91</b>	0.84	0.85	.....	5.4
14	0.36	<i>0.21</i>	0.45	0.82	<b>0.97</b>	0.91	.....	4.6
15	<i>0.17</i>	0.21	0.44	0.79	<b>0.92</b>	0.88	0.49	5.4
16	0.21	<i>0.19</i>	0.52	0.82	<b>0.97</b>	0.91	.....	5.1
17	0.23	<i>0.22</i>	0.44	<b>0.97</b>	0.95	0.66	.....	4.4
18	<i>0.16</i>	0.24	0.43	0.88	<b>0.95</b>	0.73	.....	5.9
Average	<i>0.22</i>	0.24	0.39	0.72	<b>0.82</b>	0.63	0.33	3.7

in the last column of table V, is apparently considerably smaller for the older leaves (1-5) than for the younger ones, this being due to low absolute values of the maxima rather than to high values of the minima.

### SERIES III

The third series of observations was carried out between 5:25 of August 24 and 5:00 of the following day. At this time the five oldest leaves had fallen, but fourteen new leaves had been formed and were large enough for the test. Thus this series involved leaves numbered from 6 to 32. All of

the leaves were tested, in their serial order, at the first observation, but the large number of leaves rendered the work very time-consuming, so that the interval from 5:25 to 7:30 passed before all were tested. In the other observations, beginning at hours 10, 14, 18 and 22 of the first day, and at hour 5

TABLE VI

*Indices of foliar transpiring power (entire leaf) for each of 26 leaves of a Helianthus plant, at various hours of the day, Aug. 24-25. Maxima are in black-face type and minima in italics.*

LEAF NUMBER	AUG. 24, HR. 5:25-7:30		HOUR 10	HOUR 14	HOUR 18	HOUR 22	AUG. 25 HR. 5	RATIO OF MAX. TO MIN.
	Hour	Index	Index	Index	Index	Index	Index	
6	5:25	<b>0.56</b>	0.46		<i>0.28</i>		0.30	2.0
7	5:30	0.50		<b>0.60</b>		<i>0.26</i>		2.3
8	5:40	0.55	<b>0.70</b>		<i>0.33</i>		0.39	2.1
9	5:45	<b>0.58</b>		0.53		<i>0.27</i>		2.1
10	5:55	0.62	<b>0.66</b>		<i>0.37</i>		0.35	1.8
11	5:55	<b>0.63</b>		0.51		<i>0.24</i>		2.6
12	6:00	0.45	<b>0.63</b>		<i>0.30</i>		0.30	2.1
13	6:05	<b>0.56</b>		0.52		<i>0.24</i>		2.3
14	6:10	0.57	<b>0.82</b>		<i>0.36</i>		0.48	2.3
15	6:15	0.54		<b>0.78</b>		<i>0.33</i>		2.4
16	6:15	0.58	<b>0.85</b>		<i>0.28</i>		0.51	3.0
17	6:20	0.68		<b>0.87</b>		<i>0.29</i>		3.0
18	6:30	0.59	<b>0.85</b>		0.49		<i>0.45</i>	1.9
19	6:40	0.67		<b>0.77</b>		<i>0.25</i>		3.1
20	6:40	0.51	<b>0.85</b>		<i>0.33</i>		0.50	2.6
21	6:45	0.70		<b>0.94</b>		<i>0.23</i>		4.1
22	6:50	0.86	<b>0.89</b>		<i>0.36</i>		0.52	2.5
23	6:50	0.86		<b>0.90</b>		<i>0.26</i>		3.5
24	6:55	0.65	<b>0.90</b>		<i>0.39</i>		0.55	2.3
25	7:00	<b>0.94</b>		0.87		<i>0.29</i>		3.2
26	7:10	<b>0.98</b>	0.92		<i>0.33</i>		0.51	2.9
27	7:10	0.90		<b>0.96</b>		<i>0.29</i>		3.3
28	7:15	<b>0.98</b>	0.88		<i>0.39</i>		0.81	2.5
29	7:20	<b>0.99</b>		0.90		<i>0.36</i>		2.7
30	7:20	<b>0.98</b>	0.97		<i>0.42</i>		0.78	2.3
31	7:25	<b>0.96</b>		0.66		<i>0.38</i>		2.5
32	7:30	0.92	<b>0.92</b>		<i>0.29</i>		0.81	3.2
Average		0.71	<b>0.81</b>	0.75	0.35	<i>0.28</i>	0.52	3.1

of the second, only alternate leaves were tested. Thus, at hour 10, leaves 6, 8, 10, 12, etc., were used. At the next hour (14) the remaining leaves were tested (nos. 7, 9, 11, 13, etc.), and at the fourth observation tests were repeated on the leaves used at the second. Thus this series does not present a very satisfactory picture of the daily march of transpiring power for any

one leaf, but the averages are probably reliable for all the leaves taken together. The readings were obtained in the order of the serial numbers of the leaves, beginning at the hours indicated. It took nearly an hour to complete the series of tests at each observation. The actual times of test are given (to within 5 minutes) for the first set of observations, in table VI, which presents the data obtained from this series.

From table VI it is once more clear that the average foliar transpiring power of all leaves together increases from a low night value to a maximum in the day, then falling again to the night value. With a single exception (leaf 18), all the minima of this series occur with either hour 18 or hour 22, which agrees well with the similar conclusion derived from series II for this plant. The larger values obtained for the younger leaves at the first observation (5:25-7:30) are probably related, not to the age of the leaves in question, but to the fact that these leaves were observed well after sunrise, while the oldest ones were tested before sunrise. As far as these incomplete data go, they indicate that the older leaves attain their maximum values somewhat earlier in the day than do the younger ones. In general the maxima surely all occur between hours 6 and 14, as in series II. The ranges in index values encountered in this series are generally lower than those for series II, as is indicated by the ratios of the last column of table VI, but the uncertainty regarding the actual maxima and minima renders this unsatisfactory as a generalization. It appears here again that the older leaves tend to have a narrower daily range than do the middle-aged or younger ones. The maxima for the older leaves (6-13) are relatively low, while those for the younger ones (25-32) are correspondingly high, as far as this evidence goes.

#### SUMMARY OF RESULTS OF SERIES I-III, *HELIANTHUS*

If an attempt is now made to state the relations between leaf-age and foliar transpiring power, as shown by the tests of *Helianthus* just recorded, it appears that:—

Oldest leaves have low maxima in all three series, while youngest leaves have *high* maxima for series III. The first of these statements agrees with that for *Xanthium*, series IIIa and IIIb.

The values of the minima are not here clearly related to leaf-age.

Oldest leaves show late maxima for series I, while youngest ones show early maxima in series III.

Range of index values is relatively low for oldest leaves in series II and III. Series I presents no evidence in this regard. This is the only generalization for which the available data for both *Helianthus* and *Xanthium* agree.

TABLE VII

*Data of foliar transpiring power for eighteen different leaves of Helianthus plant, obtained between 4:35 and 5:45 and between 5:50 and 6:55, of August 1, to show alteration in the index value accompanying sunrise.*

LEAF NUMBER	TIME OF OBSERVATION	INDEX OF TRANSPIRING POWER					
		Lower surface		Upper surface		Entire leaf	
		Index	Ratio	Index	Ratio	Index	Ratio
1	6:30	0.74		0.39		0.57	
2	5:05	0.47		0.36		0.41	
	6:10	0.87	1.9	0.38	1.1	0.63	1.5
	5:20	0.47		0.30		0.38	
3	6:20	0.69	1.5	0.51	1.7	0.60	1.6
	4:35	0.39		0.19		0.29	
4	6:35	0.49	1.3	0.38	2.0	0.43	1.5
	4:35	0.35		0.29		0.32	
5	6:40	0.74	2.1	0.41	1.4	0.58	1.8
	5:15	0.59		0.31		0.45	
6	6:40	0.82	1.4	0.60	1.9	0.71	1.6
	5:20	0.47		0.37		0.42	
7	6:45	0.80	1.7	0.64	1.7	0.72	1.7
	4:35	0.39		0.19		0.29	
8	6:25	0.90	2.3	0.65	3.4	0.78	2.7
	4:55	0.36		0.25		0.30	
9	6:30	0.86	2.4	0.43	1.7	0.65	2.2
	5:05	0.44		0.27		0.36	
10	6:05	0.94	2.1	0.49	1.8	0.72	2.0
	5:35	0.56		0.32		0.44	
11	5:50	0.98	1.7	0.39	1.2	0.69	1.6
	4:45	0.42		0.31		0.36	
12	6:50	0.89	2.1	0.69	2.2	0.79	2.2
	4:45	0.44		0.29		0.37	
13	6:50	0.94	2.1	0.89	3.1	0.91	2.5
	5:15	0.59		0.31		0.45	
14	5:50	0.98	1.7	0.66	2.1	0.82	1.8
	5:40	0.63		0.24		0.44	
15	6:20	0.92	1.5	0.66	2.8	0.79	1.8
	5:10	0.68		0.35		0.52	
16	5:50	0.98	1.4	0.66	1.9	0.82	1.6
	5:45	0.63		0.24		0.44	
17	6:55	0.97	1.5	0.97	4.0	0.97	2.2
	5:45	0.56		0.31		0.43	
18	6:55	0.94	1.7	0.82	2.6	0.88	2.0
Average	4:35-5:45	0.50		0.29		0.39	
	5:50-6:55	0.86	1.8	0.59	2.2	0.73	1.9

RELATION OF SUNRISE TO TRANSPIRING POWER IN *HELIANTHUS*

A special series of observations was carried out on the morning of August 1, to bring out the relation of sunrise to the sudden increase in the transpiring power of this plant, as indicated for the early morning. Each of the 18 original leaves was tested between 4:35 and 5:45 (before sunrise) and between 5:50 and 6:55 (after sunrise). Sunrise occurred about 5:50. Leaf 1 was not tested at the earlier observation. The detailed data for upper and lower leaf-surfaces, as well as those for the entire leaf, and the ratios of the earlier to the later value, in each case, are given in table VII.

The data of table VII show a pronounced increase in foliar transpiring power occurring between the two readings in every case. The shortest interval of time here represented is 10 minutes, for leaf 16, which was tested at 5:40 and again at 5:50. In this instance the index for the lower surface increased from 0.68 to 0.98, or from unity to 1.4, while that for the upper surface increased from 0.35 to 0.66, or from unity to 1.9. For the entire leaf this increase was from 0.52 to 0.82, or from unity to 1.6. These, and other similar points brought out by table VII, show clearly how very rapid is this morning increase in the index value. That the opening of stomata accompanying increase in light intensity plays an important part in the control of this increase is practically certain, but what other conditions may also be effective cannot yet be stated. It is a surprising fact that about two-thirds of the cases here recorded show the greater increase for the *upper* surface, and that only 6 leaves show the reverse (leaves 2, 5, 9, 10 and 11). This whole matter might well be made the subject of a special study, by some worker especially interested in stomata and in the influence of their opening and closing upon the power of leaves to retain water.

## CONCLUSIONS

All of the series described above agree in showing again what had been recorded for other plants before, that foliar transpiring power varies throughout the 24 hours of the day, having low values during the night and much higher values during the period of daylight. Considering the averages, representing all of the leaves of the plant, all three *Xanthium* series and series II and III for *Helianthus* agree in showing the maximum average index as occurring with the set of observations beginning with hour 10. That is, these maxima are indicated for the 11th hour of the day. Series I for *Helianthus* shows this average maximum as occurring between hour 9 and hour 10, but this slight discrepancy appears to be due rather to the exact times at which these observations were obtained than to any real physiological difference. The actual values of these average maxima, as indicated in these tests, are 0.92, 0.96 and 0.81, for the three *Xanthium*

series, and 0.86, 0.82, 0.78 and 0.85 for the four *Helianthus* series. It thus appears that the average maximum for the day is about the same for these two plant forms, which might be expected from the fact that they generally appear to thrive best in the same sort of habitat.

As to just what is to be regarded as the time of occurrence of the average minimum values, the evidence is not satisfactory, owing to the lack of more frequent observations during the night. It is clear, however, that the minima, as shown, occur for the sets of observations beginning with hour 18, or else with those beginning with hour 22 (23 for *Helianthus*, series II). Series I for both *Xanthium* and *Helianthus* failed to show this minimum, since observations at the proper hours to bring it out were not included in these series. The actual values of the average minima are likewise not as well shown by these tests as might be wished. The averages obtained are 0.15 and 0.23 for *Xanthium* (series II and III), and 0.22, 0.25 and 0.19 for *Helianthus* (series II, IIIa and IIIb). From this it appears that these two plant forms do not differ markedly in respect to their average minima of foliar transpiring power.

It is instructive to note also, in this connection, that the ratio of the day maximum to the night minimum is about the same for *Xanthium*, series III, and for *Helianthus*, series II, IIIa and IIIb, these three values being 3.5, 3.7, 3.1 and 4.5 respectively. For *Xanthium*, series II, this ratio has a much higher value, 6.4, which is not explained.

In series I for *Xanthium* the average index value passes its day maximum and then falls to a secondary, *day* minimum (hour 14), after which it once more increases. This is the only one of the six series that included observations at proper times to render such a secondary day minimum apparent, if it had been present, so that the failure of the other series to show this characteristic is not clear evidence that a corresponding minimum did not occur. This special feature of the daily march of foliar transpiring power, which has been noted before, and the conditions that control it, deserve special attention on the part of students of plant transpiration.

Both plants agree (tables III and VII, especially) in showing a very rapid increase in the value of the average index of foliar transpiring power during a short period in which sunrise occurs. This increase amounts to about 240 per cent. in the case of *Xanthium* (table III), the average value changing, in about an hour, from 0.22 to 0.75. In the case of *Helianthus* (table VII), the corresponding increase is not as rapid; it amounts to only about 90 per cent., the index value altering from 0.39 to 0.73. This may represent a true physiological difference between *Xanthium* and *Helianthus* plants growing under the conditions of these tests, but more study will be necessary before such a generalization may be made.

A study of the daily marches of the index values for the various individual leaves brings out one rather clear generalization regarding the relation

between age of leaves and their physiological behavior; namely, that the oldest one, or several, on the stem always have a low daily range of transpiring power, as far as these data show. This appears to be due to a second condition, not quite so well shown as the first, that the oldest leaves generally exhibit low maximum index values. No relation of leaf-age to daily range or to minimum value is clearly apparent throughout the various series, nor is any general relation between leaf-age and time of occurrence of the diurnal maximum or minimum to be discerned from these data.

The age relations just mentioned are to be considered as extremely tentative, nor is the lack of other such relations to be regarded as at all well shown by the somewhat meagre data of these studies. Much more work will be required before foliar transpiring power and its daily variations may be understood; enough has been done, however to show the value of this kind of study and, especially of the method of hygrometric paper here employed.

Perhaps the most important points emphasized by these tests are the great diurnal range in foliar transpiring power usually met with, together with the frequent lack of synchronism, among the different leaves of the same shoot, especially in the occurrence of the diurnal maximum. The subject of foliar resistance to water-loss by evaporation is an exceedingly complex one, and the diurnal march of the transpiring power of the leaves of a given plant must be fairly well understood before it may be stated to what degree that plant is to be regarded as drought-resistant, or xerophyllous.

*Iowa State College,  
Ames, Iowa.*

*Laboratory of Plant Physiology  
of the Johns Hopkins University,  
Baltimore, Md.*

#### LITERATURE CITED

- BAKKE, A. L. [1914], Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. *Jour. Ecol.* 2: 145-173. 1914.  
LIVINGSTON, B. E. [1913], The resistance offered by leaves to transpirational water-loss. *Plant World* 16: 1-35. 1913.  
SHREVE, EDITH B. [1914], The daily march of transpiration in a desert perennial. *Carnegie Inst. Wash. Pub.* 194. Washington, 1914.



# AN ANALYSIS OF THE CAUSES OF VARIATIONS IN THE TRANSPIRING POWER OF CACTI

EDITH B. SHREVE

## ABSTRACT<sup>1</sup>

The transpiring power of plants has been defined as the absolute transpiration rate divided by the rate of evaporation from an exposed water surface for the same period.<sup>2</sup> It has been known for a number of years that the daily march of the transpiring power of cacti differs greatly from that of non-succulents under the same conditions. In general, this difference may be expressed by saying that, while the transpiring power of non-succulents is greater during the day, that of cacti is greater by night than by day. This publication is a report upon an investigation undertaken with the hope of finding the exact nature of this behavior in cacti and its accompanying phenomena, and of establishing, if possible, relations of cause and effect. The material used was *Opuntia versicolor*. The following is an outline of the report:

- A. Abstract.
- B. Introduction.
- C. Relation of transpiring power ( $T/E$ ) to various factors.
  - 1. Desiccating power of the soil.
  - 2. Water-content of tissues.
  - 3. Absorbing power of roots.
    - a. Derivation of secondary absorbing power ( $A/T$ ).
- D. Inter-relation of  $T/E$ ,  $A/T$ , and water-holding capacity of tissues.
  - 1. Measurement of  $T/E$  and  $A/T$  under different sets of environmental conditions.
  - 2. Measurement of water-holding capacity of tissues under different sets of environmental conditions.
- E. Effect of variations in water-holding capacity of tissues on  $T/E$  and  $A/T$ .
  - 1. Statement of theory.
  - 2. Possible influence of the water-holding capacity of tissue on its evaporating power.
  - 3. Stomatal apertures under typical and atypical environmental conditions, and their theoretical relation to the water-holding capacity of the tissue.
  - 4. General statement of theory of the effect of water-holding capacity on  $T/E$ .
- F. Preliminary investigation of some of the causes of the variations in water-holding capacity of the tissue.

<sup>1</sup> The manuscript of this paper was received June 15, 1916. This abstract was preprinted, without change, from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 2, no. 3, August, 1916.

<sup>2</sup> It will be noted that the word *power* is not thus used in its exact physical sense, since the factor of distance does not appear.

1. Theory of the influence of acid.
2. Acidity variations compared with variations in  $T/E$ ,  $A/T$ , and water-holding capacity of the tissue.

#### F. Conclusion.

The main conclusions drawn from the work are as follows:

1. The transpiring power of *Opuntia versicolor* is greatly influenced by light intensity, air temperature, water-content of tissues, and available soil moisture; these factors clearly exert their influence indirectly through their action upon some internal process.

2. Variations in the rate of water intake by the roots occur, and they are apparently independent of variations in transpiration. Variations in water intake by the roots are due, on the one hand, to variations in soil retentivity, and on the other, to variations within the plant itself. The latter may be further subdivided into variations in absolute transpiration rate and in water-absorbing power of the tissues. The variations in soil retentivity may be reduced to zero, for experimental purposes, by the use of water cultures or supersaturated soil; then the absolute water intake divided by the absolute transpiration for the same periods gives quantities whose variations can be traced neither to soil retentivity nor to transpiration changes. This quantity ( $A/T$ ) is given the name "secondary absorbing power." It is found to vary in a direction which is always opposite to the variations in  $T/E$  for the same period, that is,  $T/E$  is greater by night and  $A/T$  by day.

3. The water-holding capacity of cylinders cut from internal tissue is found to be less at night than during the day. It parallels the behavior of  $A/T$  under all the several environmental conditions used in the experiments.

4. The stomata are, in general, closed during the day and open at night. Some evidence appeared that a decrease in  $T/E$  preceded stomatal closing.

5. The theory is advanced that the variations in transpiring power and in secondary absorbing power are due to variations in the water-holding capacity of internal tissue. In the case of the transpiring power, the changes in water-holding capacity act indirectly by causing the closing of the stomata, and directly by resisting the evaporative power of the air.

6. The source of energy for this resistance to the evaporative power of the air may be traced to the imbibitional forces of the cell walls, and of the emulsoid colloids of the protoplasm, and hence to surface tension forces.

7. The effects exerted by light intensity and air temperature, especially when their duration is considered, indicate that the variations in water-holding capacity of tissue are due, at least in part, to chemical changes brought about by metabolic processes. Under "typical" conditions, a high water-holding capacity is accompanied by a low acidity and vice versa. However, certain exceptions occurring under controlled conditions indicate that the relation is not so simple as the influence of mere changes in hydrogen-ion concentration. The regular accumulation and disappearance of many

substances within the plant must be considered, not only in the light of their individual influences on the water-holding capacity but also in that of their combined effect.

8. In a former paper the author described well-defined autonomic movements in cacti, which could be accounted for by changes in the water-content of the plant. These changes in water-content are of such a nature that the difference between absorption and transpiration is positive for the day and negative for the night. The facts brought out in this paper show that the cause of these movements may be further traced to changes in the water-holding capacity of internal tissue.

9. As desiccation progresses a cactus loses less and less water by transpiration until a point is reached where the total loss for 24 hours is almost zero. There is a small loss during the daylight hours which is frequently entirely balanced by a gain at night. Only about one-tenth of this gain can be accounted for by hygroscopic properties of the spines.

10. The water absorbing capacity of internal tissue from plants which have been without water for 6 months is about five times as great as that of tissue from plants which have had enough water. This is true only if the absorbing capacity be based upon wet weight.

11. The interpretation of paragraphs 9 and 10 may be as follows: as the plant loses water during a drought period the total mass has an increasing hold upon its water, until a balance is reached, when the vapor pressures within and without the plant are nearly equal, even a gain in water content taking place at night when the humidity is higher than on the preceding day. The increased absorbing capacity of the tissue would also tend to increase the pull of the roots on the soil water, and thus perhaps change the amount of available soil water, even though the water content of the soil is not raised.

12. The ability of the cactus to withstand long periods of drought is thus traced to its power to hold water within its tissue against the evaporative forces of the air. It has been found by various workers that non-succulent plants growing in the same desert region as these cacti show a decrease in their transpiring power in the forenoon, while the evaporative force of the air is still increasing, and the cause for this has been attributed to a partial drying out of the tissue of the plant, causing the water films to retreat within the pores of the cellulose and there, obeying the laws of surface tension, to acquire a decreased vapor tension. The present investigation has shown that the tissue of this *Opuntia* has a changing water-holding capacity when there is so much water present that there can be no retreating into pores, which suggests the hypothesis that this change is the effect of various chemical changes on the absorbing power of colloidal gels. It seems not unreasonable to suppose that the lowering of the transpiring power during the day, in non-succulents, may be due to such changes in the water-holding capacity

of the tissue. The smaller bulk of tissue in non-succulents would have less effect upon transpiring power, so that their transpiring power for the day might still be greater than for the night. No tests were made for non-succulents; this hypothesis for their behavior is offered only as a suggestion.

## INTRODUCTION

The water requirements of plants may be divided into two general classes, the one being the need for replacement of loss by transpiration, the other the necessity for water to be retained within the tissues for the maintenance of turgidity and growth. For the majority of plants the amount of water retained within the tissues is only a small portion of the total water requirement. Cacti, however, retain proportionally more water than do other plants; so that, for any given year, a growing cactus uses for internal processes a larger percentage of its total water consumption than does a non-succulent, even in a case where the two consume the same total amount.

Both types of plants fulfill their water requirements by intake through their roots, but if this means of supply becomes inadequate, either by reason of an increase in the transpiration rate, or a decrease in the available soil water, the cactus can remain alive for weeks and months while the non-succulent usually dies within a few hours or days.

An analysis of the external factors which influence the water relations of plants has recently been made by Livingston and Hawkins,<sup>3</sup> who obtain an aridity formula,  $D_e = d_a \times d_s$ , where  $d_a$  is the desiccating power of the aerial surroundings,  $d_s$  the water attracting power of the soil, and  $D_e$  the total water removing power of the environment as a whole. This  $d_a$  is the familiar " $E$ ", a summation of the results of the various evaporative forces of the air as registered by a standardized atmometer. When the absolute transpiration of a plant is divided by this  $E$  for the given period the quotient,  $T/E$ , is termed, by the authors just cited, "the transpiring power of the plant." If  $T$  and  $E$  are reduced to the same units any deviation from unity which is shown by the quotients must be due to causes other than the direct effects of the evaporative power of the air. In the same way, the absolute absorption at the roots, divided by the power of the soil to deliver water to the roots, gives a quotient whose deviations from unity represent changes in absorption due to causes other than those originating in the soil. The quotients  $T/E$  and  $A/S$ , then represent changes due to internal causes, in so far as they deviate from unity. I shall henceforth use the terms transpiring power and absorbing power with the meaning given them by Livingston and by Livingston and Hawkins; and by absolute transpiration and absolute absorption I shall mean the total amount lost or absorbed during a given period.

<sup>3</sup> Livingston, B. J., and Lon A. Hawkins. The water relation between plant and soil. Carnegie Inst. Wash. Pub. 204: 3-48. Washington, 1915. Page 21.

It has not yet been practicable to measure the numerator and denominator of these fractions in the same units. But it is, nevertheless, possible to determine the changes in transpiring or absorbing power which are due to internal conditions, for wherever the graph, which is made from a number of successive quotients, deviates from a horizontal line, there appear the results of the influence of internal forces.

Various workers<sup>4</sup> have found that the graph of transpiring power is not a horizontal line. In the majority of plants it approximates a horizontal line under the usual night conditions, but during the day it rises and then falls, in a fashion which roughly parallels the graph of *E*. Although the internal forces acting here are doubtless due to free energy absorbed by the leaf, they are just as truly internal, since it is the raised temperature of the internal tissues which increases the vapor pressure within the intercellular spaces and thus accelerates transpiration.

Non-succulent plants growing under desert conditions have been found to give a graph of transpiring power which rises steeply in the morning and then either becomes horizontal or descends, while the evaporation graph is still ascending and the light intensity continues to increase. The cause of this "check" in transpiring power has been ascribed by Livingston and Brown<sup>5</sup> to incipient drying of the foliar cells due to "a fall below unity of the ratio of possible water supply to water loss," when the water films gradually retreat into the pores of the cellulose, decreasing the amount of exposed evaporating surface and increasing the surface tension.

On the other hand, the graph of transpiring power of cacti has been found<sup>6</sup> to approximate a straight line during the day time and to ascend at night. It seems reasonable that the transpiring power of a plant should be greater by day than by night since the plant is absorbing heat energy during the daylight hours; but in order to account for the energy necessary to an increased night rate there must be postulated either an internal source of energy or it must be supposed that the energy available during the day is by some means prevented from being used for evaporation and hence must be diverted to other channels.

I have made a study of the transpiration of *Opuntia versicolor* with the hope of adding to our knowledge of the mechanism which controls the transpiring power of cacti, of discovering the source of energy needed for

<sup>4</sup> Livingston, B. E., The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Wash. Pub. 50. Washington, 1906. (Also many other papers; for references see Livingston and Hawkins [1915].)

Lloyd, F. E., The physiology of stomata. Carnegie Inst. Wash. Pub. 82. Washington, 1908.

Shreve, F., A montane rain-forest. Carnegie Inst. Wash. Pub. 199. Washington, 1914.

Bakke, A. L., Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. Jour. Ecol. 2: 145-173. 1914.

Shreve, E. B., The daily march of transpiration in a desert perennial. Carnegie Inst. Wash. Pub. 194. Washington, 1914.

<sup>5</sup> Livingston, B. E., and W. H. Brown, Relation of the daily march of transpiration to variations in the water-content of foliage leaves. Bot. Gaz. 53: 309-330. 1912.

<sup>6</sup> Livingston, B. E., Relative transpiration in cacti. Plant World 10: 110-114. 1907.

their high nocturnal transpiring power, and of describing the manner in which they tide over long periods of drought.

The work has been carried on at the Desert Laboratory, at Tucson, Arizona. I wish to express, here, my gratitude to Dr. D. T. MacDougal both for the use of the laboratory and its facilities and for several very helpful suggestions.

## RELATION OF TRANSPIRING POWER TO VARIOUS FACTORS.

The influence of the desiccating power of the soil upon transpiring power will be first considered. Since the water-content of a given kind of soil is roughly proportional to the power of the soil to deliver water to the roots, I have used large differences in water-content of the soil and have made no actual measurements of its desiccating power.

### *Experiment 1*

*Object:* A preliminary examination of the absolute transpiration and of the transpiring power of *Opuntia versicolor* under widely different conditions of water-content of soil.

*Material:* Seven potted plants from 3 to 10 years old were used. No. 2 was raised in the green-house and was 4 years old at the time of the experiment. The other plants were transferred to pots from their natural position in the open about a year before they were used. With the exception of Nos. 2 and 3 all the plants spent their entire lives in the open. No. 3 was placed in the green-house just after it was potted and during the year acquired a long, somewhat attenuated joint. This joint and all of the joints of No. 2 were longer and bore smaller spines than the joints from the plants which remained in the open.

*Method:* The plants were sealed, in the Ganong type of aluminum shells, on Oct. 22, 1913, and were left sealed, without the addition of water, until Jan. 21, 1914. The sealing at the top of the shell was made by means of paste-board covers thickly coated with a plasticine which contained no sulphur. Rubber stoppers were bound loosely about the woody base of the main stem in order to prevent the plasticine from coming in contact with the plant tissues. A final dust-proof sealing was made by covering the plasticine and stopper with tin-foil. The shells were two sizes larger than the pots, so that there was an air-space between pot and shell. No. 2 was given water immediately before it was sealed, the others had received no water for three days previous to the sealing.

Transpiration was measured by loss of weight from the sealed pots, and the evaporative power of the air was measured by loss of weight from porous cup atmometers of the Livingston cylindrical type. The atmometers were always run in duplicate and the cups were alternately changed every 24

hours for newly standardized ones. In this way each cup was in use 48 hours. All cups were standardized by *The Plant World* both before and after use. The evaporation losses have been reduced to standard by the use of the coefficients furnished by the standardization; consequently all evaporation numbers which appear in this paper are comparable. However, the water losses from the various plants have not been reduced to unit area and therefore the transpiration rates from different plants are not comparable in actual amount. Of course the different transpiration rates from the same plant may always be compared unless considerable growth took place between observations. Individual plants will retain the same numbering throughout the experiments to be described below.

*Results:* The data are recorded in table 1. In the column headed  $T$  appear the average number of grams of water lost by the whole plant per hour, during the period indicated.  $E$  represents the rate of loss from the atmometer.  $T/E$  is obtained by dividing each quantity in the first column by the corresponding one in the second. In the tables as well as in the text, letters in parenthesis denote the light conditions: dk denotes a period of continuous darkness, lt a period of continuous bright sunlight, and sh indicates partial cloudiness or shade within the laboratory.

An examination of table 1 shows the following: For moderately wet soil. (October observations) the transpiring power ( $T/E$ ) is greater for the night than for the day, while the absolute transpiration is generally greater during the day. After 3 months of desiccation the absolute loss by transpiration for the night has been replaced by a slight *gain*, with two exceptions; but the absolute transpiration for the day has, in general, not been greatly reduced. After this amount of desiccation the transpiring power is, in all cases, greater for the day than for the night. A drooped and shrunk condition of the joints in January gave evidence that the ratio of supply of water to loss had been less than unity for some time. Between October and January the desiccating power of the air had decreased about 50 per cent. and consequently it is best to withhold conclusions as to the influence of this factor until the test is repeated with similar conditions of light, temperature and humidity existing throughout the experiment.

The facts suggested, but not necessarily proved, by experiment 1 are: (1) The amount of water in the soil influences the absolute transpiration rate and the transpiring power; (2) plants Nos. 2 and 3 indicate that the amount of water in the tissues, as well as in the soil, may influence the nocturnal transpiring power; (3) after plants have been desiccated for 3 months an actual gain at night may appear, amounting to as much as the total loss for the preceding day.

Before undertaking the repetition of this experiment it seemed advisable to investigate in more detail the gain in weight at night. Experiment 2 was carried out for this purpose.

TABLE 1  
*Absolute and relative transpiration of plants of Opuntia versicolor in (1) wet soil and (2) dry soil. All amounts are grams per hour.*  
*(Experiment 1)*

(Experiment 1)

DATE	TIME	ABSOLUTE TRANSPIRATION ( $T$ )								TRANSPIRING POWER ( $T/E$ )								EVAPORATION ( $E$ )
		No. 2	No. 5	No. 7	No. 9	No. 3	No. 6	No. 8	No. 2	No. 5	No. 7	No. 9	No. 3	No. 6	No. 8			
Oct. 22.....	5 p.m.																	
	7 a.m. (dk) <sup>a</sup>	0.089	0.007	0.015	0.007	0.109	0.020	0.010	0.26	0.023	0.045	0.020	0.32	0.058	0.029	0.343		
Oct. 23.....	6 p.m. (lt)	0.164	0.027	.....	0.027	0.100	0.036	0.009	0.11	0.018	.....	0.018	0.07	0.024	0.006	1.480		
Jan. 20.....	6 p.m.																	
Jan. 21.....	7 a.m. (dk)	0.029	<sup>b</sup>	0.000	<sup>c</sup>	0.059	<sup>d</sup>	<sup>e</sup>	0.14	<sup>b</sup>	0.000	<sup>c</sup>	0.28	<sup>d</sup>	"	0.210		
	5 p.m. (lt)	0.070	0.027	0.010	0.025	0.070	0.020	0.010	0.10	0.039	0.015	0.037	0.10	0.029	0.015	0.680		

<sup>a</sup> The letters dk denote darkness and lt, light.

<sup>a</sup> The letters dk denote darkness and lt, light.

<sup>b</sup> Total gain, night, 0.20.

<sup>c</sup> Total gain, night, 0.20.

<sup>d</sup> Total gain, night, 0.11.

<sup>e</sup> Total gain, night, 0.12.

*Experiment 2, part I*

**Object:** To show whether or not the gain in weight at night, as shown by plants in experiment 1, is caused by absorption of water from the air.

**Material:** Plant No. 3, which had been sealed and left without water for 4 months. For several nights previous to this experiment it had shown a gain in weight of 0.1 gram and over.

**Method:** The apparatus used, which has been previously fully described (E. B. Shreve [1914]) consists of a large bell-jar fitted with a base containing grooves for a mercury seal. Through an air-tight joint at the top a dew-point apparatus is admitted in such a manner that the dew-point of the air within may be found without disturbing conditions inside the jar. At the beginning of the experiment the plant was weighed and placed under the jar. A shallow dish containing wet filter paper was also weighed and placed beside the plant, in order to keep the humidity within the jar from falling too low. To avoid the possibility of condensation of water on the plant or

TABLE 2

*Comparison of night gain in weight of a plant of *Opuntia versicolor*, with the weight of water vapor lost from the air surrounding the plant. (Experiment 2, part I)*

TIME	DEW-POINT OF AIR	WATER GAINED BY AIR	WATER LOST BY FILTER PAPER	WATER LOST BY PAPER AND NOT GAINED BY AIR	WEIGHT GAINED BY PLANT
	<i>deg. C.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
5:30 p.m. ....	2.8				
8:15 a.m. ....	7.8	0.026	0.340	0.31	0.30 <sup>+</sup>

pot, care was taken that the air within the jar was never near the saturation point for any temperature which might occur. As soon as the jar was sealed the dew-point was taken. At the close of the experiment the dew-point was again obtained, just before the jar was lifted. The plant and the dish containing filter paper were then again weighed. The difference between the weight of water vapor in the known volume of the bell-jar, at the beginning and at the end of the experiment, was next found (by means of the Smithsonian meteorological tables) and this amount applied as a correction to the amount lost by the filter paper. This corrected loss from the paper was then compared with the amount gained by the plant. The entire system was kept in a cool dark room during the experiment.

**Results:** From table 2, which gives the data for this experiment, it is seen that the amount of water vapor lost from the air and filter paper is 0.31 g. while the gain in weight by the plant is 0.30 g. Since these two numbers agree within the limits of experimental error, we may conclude that the gain in weight was due to absorption of water vapor from the air.

*Experiment 2, part II*

The question at once presents itself, whether or not the hygroscopicity of the spines may be responsible for the absorption of the water vapor here shown. MacDougal<sup>7</sup> found that a small plant of *Echinocactus wislizeni*, which had been desiccated for 2 years, gained 2 grams in 6 days, when kept in the dark at an equable temperature of about 15.5°C. (60°F.) and with a relative air humidity of from 80 to 90 per cent. He attributed the cause of gain to hygroscopic absorption of water by the spines.

In order to test this question for the present case, all of the larger spines were cut off at the base and placed beside the plant under the bell-jar, and part I of this experiment was then repeated. During the night the plant gained 0.30 g. and the spines made a gain of 0.02 g. During the following day the spines lost 0.02 g. Thus only a very small part of the water absorbed by the plant can be attributed to the hygroscopicity of the spines.

Experiment 2 has thus shown that the gain in weight at night, shown in experiment 1, is due to the absorption of water vapor and that the spines are responsible for this gain in only a very small degree.

*Experiment 3*

*Object:* The measurement of absolute transpiration and of transpiring power under three different conditions of soil water-content and tissue water-content, with light intensity, air temperature and air humidity similar throughout the experiment.

*Material:* Plants Nos. 2, 3, 9, 14, and 15. No. 2 was raised from seed in the green-house. Nos. 3 and 9 were transplanted from the open, but at the time of the experiment they had large joints which had grown in the green-house. Nos. 14 and 15 were kept in the open continuously after they were transplanted from the soil. None of these plants had suffered from drought for at least nine months.

*Method:* On Oct. 5 all plants were given a small amount of water and the pots were sealed, exactly as in experiment 1. During the day the plants were in the green-house, where direct sunlight fell on them, and at night they were placed in a dark room where the temperature was from 10° to 7.2°C. (50°-45°F.) After the close of the 5 p.m. readings on Oct. 6, the pots were unsealed, taken out of the aluminum shells, and left in the green-house until Oct. 27, no water being added in the meantime. On the last date the plants showed shrunken tissues and drooped joints, the characteristics of a condition which I shall henceforth designate as "desiccated." On this date the pots were sealed again, still without addition of water, and weighings

<sup>7</sup> MacDougal, D. F., *The water-balance of succulent plants*. Carnegie Inst. Wash. Pub. 141. Washington, 1919.

were begun. On Oct. 29 the pots were again unsealed and the soil was well soaked with water. Irrigation was continued daily until Nov. 1, when the tissues appeared turgid but not as plump as on Oct. 5. On Nov. 1 the pots were once more sealed, with the soil moderately dry, and weighings were again made for night and day losses. Thus, an attempt was made to obtain transpiration rates when the water-contents of the tissues and those of the soil were high, low, and medium. Since the pots were left unsealed during the different parts of the experiment desiccation went on much faster than during the course of experiment 1, with the result that the climatic conditions were much more similar in the different parts of this experiment than they were in the two parts of the former experiment.

The water-content of the soil was found by means of samples taken from the pots with a 1.5 cm. cork-borer. These samples were placed in covered weighing bottles, weighed and then dried to constant weight in an electric oven at 95°C. The amounts, given in table 3, are expressed in grams of water per gram of dry weight. The method used for the determination of the water-content of the tissues is described on a succeeding page.

*Results:* The data given in table 3 are shown in graphical form in figure 1. The dots on the graphs represent the center of the time period in each case. When the water-content of soil and tissues is high the transpiring power ( $T/E$ ) is much greater at night than during the day. After extreme desiccation  $T/E$  has greatly decreased both for day and night, the reduction for the night, however, being much greater than for the day, so that the amount for the day is now equal to, or greater than the amount for the night. On Nov. 1-4, when the plants were at least two-thirds recovered from the effects of drought, the value of  $T/E$  for the night has increased to approximately the same value it had on Oct. 5-6 (i.e., before desiccation began); but the day rate has become less, for most of the plants, than it was under the extreme conditions of desiccation.

While conclusions deduced from the absolute transpiration rates are, as a rule, of little value because they do not take into account the evaporative power of the air, still it is worth while noting that on Nov. 1-4 even the absolute transpiration is greater at night than during the day.

This experiment confirms the tentative conclusions which were derived from experiment 1, making it very evident that the available supply of water influences the value of the ratio  $T/E$ . But, in the case of a plant of this kind, which contains a large amount of water in its tissues, the question naturally arises, whether the lowering of the amount of absolute absorption, which is caused by the increasing desiccating power of the soil, affects the transpiring power directly, or indirectly by a cumulative effect on the tissues. Plants Nos. 2 and 3 in experiment 1 have already given evidence that the water-content of the tissues influences the value of  $T/E$ .

TABLE 3  
*Absolute transpiration and transpiring power of 5 plants, under three conditions of water-content of soil and tissues. (Experiment 3)*

DATE	TIME	ABSOLUTE TRANSPIRATION ( $T$ ) GRAMS PER HOUR						TRANSPIRING POWER ( $T/E$ )						WATER-CONTENT OF SOIL, GRAMS PER GRAM OF DRY WEIGHT						WATER-CONTENT OF TISSUE, GRAMS PER GRAM OF DRY WEIGHT					
		No. 2	No. 3	No. 9	No. 14	No. 15	No. 2	No. 3	No. 9	No. 14	No. 15	EVAPORATION ( $E$ ) GRAMS PER HOUR						No. 2	No. 3	No. 9	No. 14	No. 15	No. 4	No. 1	No. 16
Oct. 5.....	5 p.m.																								
Oct. 6.....	7 a.m. (dk) <sup>a</sup>	0.18	0.21	0.14	0.06	0.08	0.40	0.47	0.31	0.13	0.18	0.450	0.225	0.215	0.202	0.171	0.173	0.25	0.3	0.23	0.6	0.17	0.7		
	5 p.m. (lt)	0.37	0.32	0.33	0.18	0.18	0.29	0.25	0.26	0.14	0.14	1.28													
Oct. 27.....	5 p.m.																								
Oct. 28.....	7 a.m. (dk)	0.006	0.002	0.017	0.005	0.000	0.010	0.003	0.029	0.008	0.000	0.580	0.042	0.014	0.021	0.010	0.014	8	8	10	2	10	8		
	5 p.m. (lt)	0.015	0.016	0.036	0.015	0.027	0.013	0.014	0.031	0.013	0.024	1.14													
Nov. 1.....	6 p.m.																								
Nov. 2.....	7 a.m. (dk)	0.110	0.225	0.195	0.096	0.071	0.244	0.500	0.433	0.214	0.157	0.450	0.124	0.112	0.119	0.114	0.121	14	8	16	1	15	9		
	6 p.m. (lt)	0.025	0.025	0.042	0.024	0.029	0.036	0.036	0.061	0.035	0.042	0.69													
	7 a.m. (dk)	0.111	0.114	0.092	0.050	0.067	0.236	0.242	0.196	0.106	0.142	0.47													
Nov. 3.....	12 noon (dk)																								
	6 p.m. (lt)	0.023	0.023	0.036	0.003	0.026	0.029	0.029	0.015	0.004	0.033	0.79													
Nov. 4.....	9 a.m. (dk)	0.097	0.120	0.093	0.045	0.069	0.216	0.266	0.206	0.100	0.153	0.45													

<sup>a</sup> The letters dk denote darkness and lt, light.

In order to separate the effects of the desiccating power of the soil from those due to the water-content of the plant the next experiment was performed.

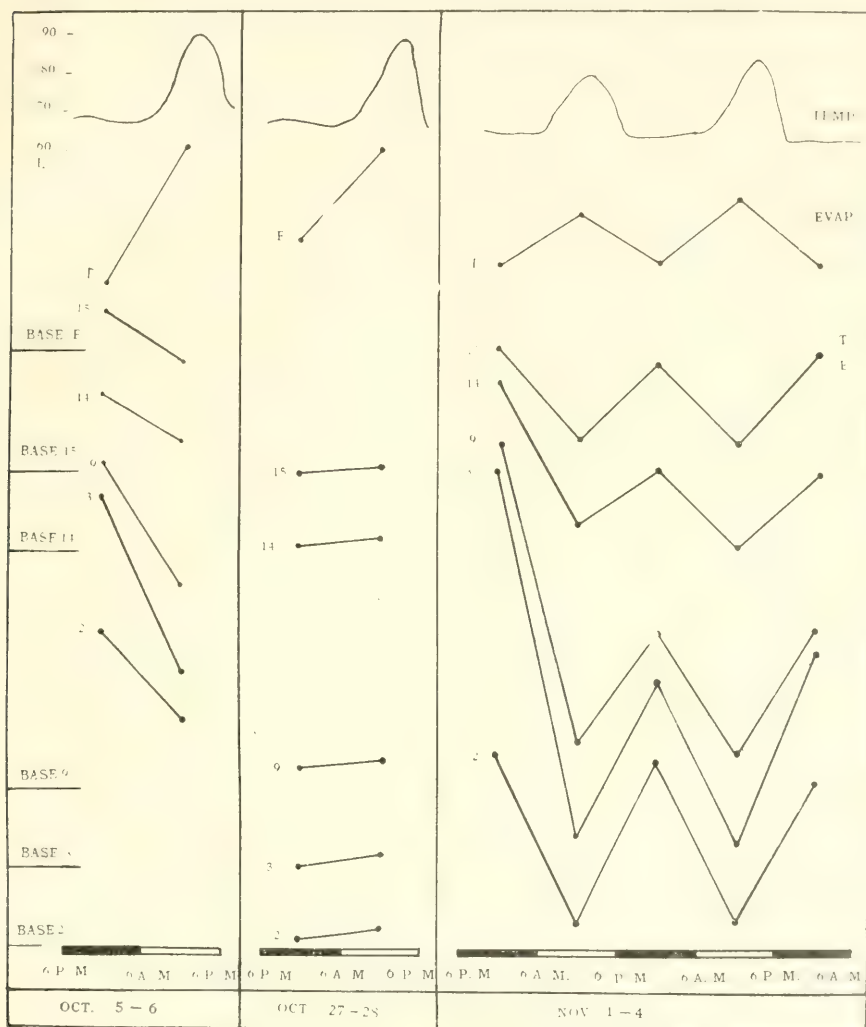


FIG. 1. Transpiring power of five plants under three different conditions of water-content of soil and tissues. (See experiment 3, table 3.)

### Experiment 4

*Object:* The measurement of the diurnal and nocturnal march of absolute transpiration and of transpiring power, under several conditions of water-content of tissues, when no intake of water by the roots is possible.

*Material:* Three potted plants, Nos. 2, 9, and 20; and two cut branches, a and b, from an adult bush. Nos. 2 and 20 were raised in the green-house. No. 20 was in situ in the open up to the time of the beginning of the experiment.

*Method:* The whole plants were taken from their soil with as little disturbance of the roots as possible. The roots were washed and lightly dried on filter paper and then placed in glass cups, the upper parts being in the air.

TABLE 4

*Absolute transpiration and transpiring power of potted plants and severed branches, under different conditions of water-content of tissues. No water intake at roots possible. Data expressed as grams per hour. (Experiment 4, figure 2.)*

		TRANSPIRATION ( $T$ )					TRANSPIRING POWER ( $T/E$ )					EVAPORATION ( $E$ )
DATE	TIME	Plants			Branches		Plants			Branches		
		No. 2	No. 9	No. 20	a	b	No. 2	No. 9	No. 20	a	b	
Nov. 16.	6:44 p.m.											
	7:23 a.m. (dk) <sup>a</sup>	0.062	0.059	0.297	1.171	0.517	0.155	0.147	0.742	2.927	1.292	0.40
	10:17 a.m. (lt)	0.024	0.030	0.018	0.230	0.103	0.045	0.057	0.034	0.434	0.194	0.53
Nov. 17.	1:31 p.m. (lt)	0.055	0.044	0.116	0.188	0.150	0.059	0.048	0.126	0.203	0.163	0.92
	3:53 p.m. (lt)	0.222	0.162	0.066	0.234	0.120	0.206	0.150	0.061	0.217	0.111	1.08
	6:3 p.m. (lt)	0.192	0.114	0.014	0.040	0.000	0.213	0.127	0.016	0.044	0.000	0.90
	9:17 p.m. (dk)	0.156	0.078	0.240	0.011	0.000	0.372	0.186	0.571	0.026	0.000	0.42
Nov. 18.	12:8 a.m. (dk)	0.114	0.108	0.474	0.080	0.066	0.278	0.263	1.156	0.195	0.161	0.41
	3:22 a.m. (dk)	0.228	0.202	0.324	0.150	0.174	0.507	0.449	0.720	0.333	0.387	0.45
	8:20 a.m. (dk)	0.150	0.164	0.098	0.050	0.128	0.375	0.410	0.245	0.125	0.320	0.40
	12:29 p.m. (lt)	0.024	0.037	0.037	0.114	0.060	0.041	0.064	0.064	0.196	0.103	0.58
	1:10 p.m.											
Nov. 22.	5:30 p.m. (lt)	0.023	0.009	0.028	0.062	0.034	0.026	0.010	0.031	0.069	0.038	0.89
	9:2 p.m. (dk)	0.000	0.008	0.006	0.017	0.000	0.000	0.018	0.014	0.039	0.000	0.44
	12:1 a.m. (dk)	0.010	0.000	0.000	0.013	0.010	0.023	0.000	0.000	0.029	0.023	0.44
	2:54 a.m. (dk)	0.014	0.004	0.000	0.010	.....	0.030	0.008	0.000	0.021	.....	0.47
Nov. 23.	7:37 a.m. (dk)	0.032	0.043	.....	0.032	0.013	0.073	0.098	.....	0.073	0.029	0.44
	9:27 a.m. (lt)	0.016	0.022	0.015	0.057	0.057	0.040	0.055	0.037	0.142	0.142	0.40
	12:15 p.m. (lt)	0.030	0.020	0.051	0.117	0.076	0.048	0.032	0.081	0.185	0.121	0.63
Nov. 24.	6:36 p.m. (lt)	0.012	0.025	0.015	0.058	0.034	0.014	0.029	0.018	0.068	0.040	0.85
	7:34 a.m. (dk)	0.044	0.028	0.019	0.046	0.017	0.098	0.062	0.042	0.102	0.038	0.45

<sup>a</sup> The letters dk denote darkness and lt, light.

Air-tight seals were then made, just as in the former experiments, and the cups were wrapped to exclude light from the roots. The cut branches were sealed at their open ends with grafting wax and tin-foil. Plants and branches were then immediately weighed and weighings were continued at intervals of three hours from Nov. 16, 6 p.m. to Nov. 18, noon. After this they were placed, intact, in the green-house and left there without water until Nov. 22, when shrunken tissues and drooped joints plainly indicated a lowered water-content of the tissues. Weighings were repeated Nov. 22-24.

*Results:* Data appear in table 4 and figure 2. From an examination of the results the following facts appear: (1) When the tissues are turgid

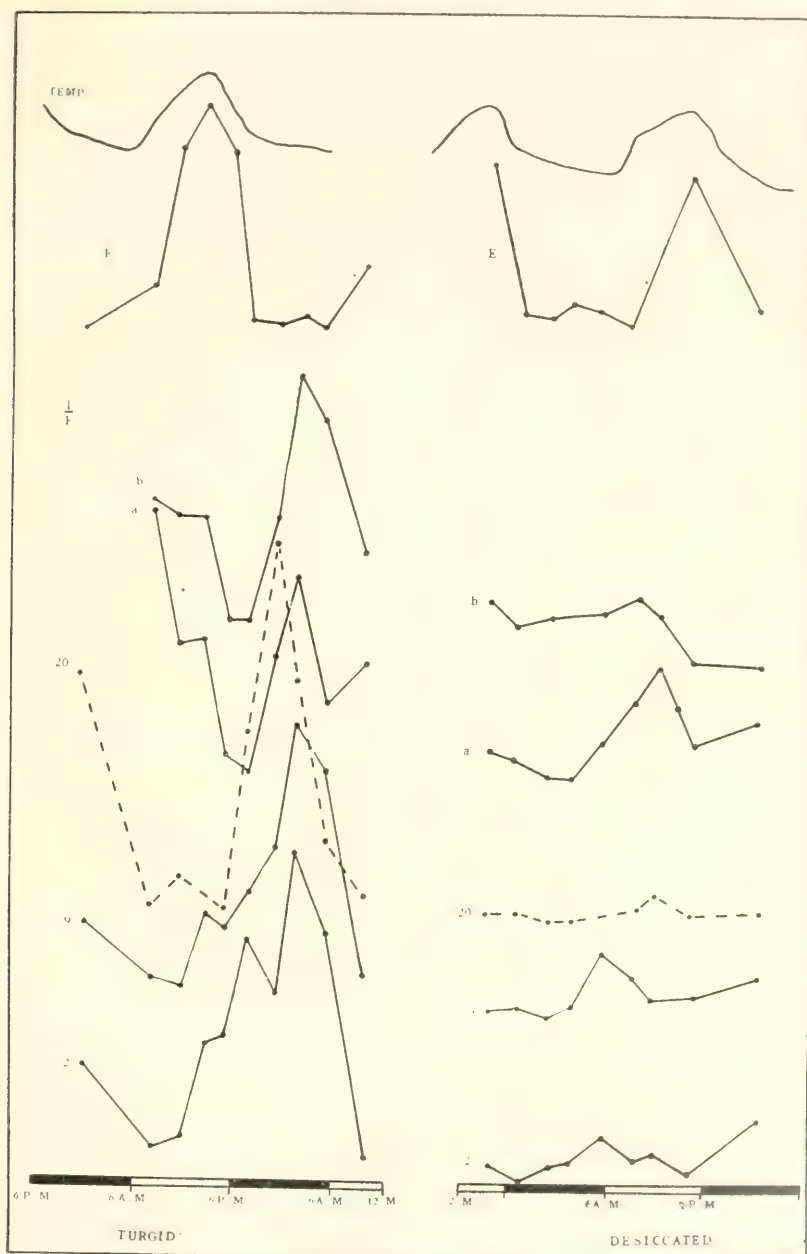


FIG. 2. Transpiring power of two branches (a and b) and three potted plants, (1) with turgid tissues and (2) with desiccated tissues. (See experiment 4, table 4.)

the value of  $T/E$  is less for the day than for the night; (2) after desiccation, the day and night values of  $T/E$  both decrease, the latter more than the former; (3) after desiccation  $T/E$  for the day was greater than for the night, in two cases.

Comparison of the results of this experiment with those of experiments 1 and 3 shows conclusively that it is the water-content of the tissues which influences the transpiring power and that the desiccating power of the soil is responsible only indirectly, as it affects the water-content of the tissues. The changes in transpiring power between day and night take place even when no water intake at the roots is possible, and it therefore follows that changes in absorbing power cannot be the cause of the variations in transpiring power.

However, it is quite possible that there may be changes in absorbing power brought about by the same internal phenomena that cause the changes in transpiring power. Consequently, in the next experiment the absorption rate is investigated and compared with that of transpiration. In order to obtain the changes in absorption rate which are due to internal causes alone, the factor  $d_s$ , the desiccating power of the soil, has been eliminated, in one case by keeping the roots directly in water, and in another case by having the soil in the pots completely saturated. Thus the resistance of the soil is reduced to zero and all changes occurring in the absolute absorption must be due to internal causes; that is, absolute absorption here becomes identical with absorbing power.

#### *Experiment 5*

*Object:* The comparison of the water lost by transpiration with the water absorbed by the roots, both for day and for night.

Two very different methods were employed for the determination of the absorption by roots and this experiment is therefore divided into two parts, each part employing a different method.

*Material, part I:* Plants Nos. 13 and 14. No. 13 was about 4 years old at the time of the experiment and until June 1, 1914, had spent its entire life in the open. On that date it was cut off just above the roots and placed with the lower part of its stem in a bottle containing water. Small roots appeared within the first week and by June 27 a large, fibrous root-system had been formed in the water. No. 14 was less than 2 years old at the time of the experiment; it had been in the green-house during its entire life, where it had been raised from seed. On June 4 it was taken from the soil, with its fleshy roots intact, and the roots were placed in a bottle of water similar to the one used in the other case. The fibrous roots were largely destroyed in this transfer but a new healthy system developed within two weeks. The water in the bottles was changed once a week. (Several other plants were started in water but bacterial action on the roots rendered them unfit for use.)

*Method, part I:* The plants were sealed in their bottles in the following manner: The bottle was completely filled with water and a 3-perforate rubber stopper was cut into two pieces and then forced into place around the woody base of the plant, so as to fit tightly in the neck of the bottle. One of the three holes was filled by the stem of the plant, the second by a thermometer, and the third by a burette of narrow diameter, graduated to  $\frac{1}{20}$  cc. The system was air-tight, with the water in the burette standing at a convenient height above the stopper.

The loss by transpiration was found by direct weighing of the bottle and its equipment. As formerly, cylindrical porous cup atmometers were used to measure the evaporative power of the air. In order to measure the water intake at the roots the height of the liquid in the burette was read at the beginning and end of each observation period, and the difference between the two readings was corrected for expansion or contraction due to temperature changes<sup>8</sup>.

*Material, part II:* Three plants, Nos. 30, 31, and 34, between 6 and 10 years old. They were transplanted from the open about 8 weeks before the beginning of the experiment and were placed in well-drained, galvanized iron cylinders, where they had become well established by the time of the experiment.

*Method, part II:* The method used has been described in the paper last cited (E. B. Shreve [1915], pp. 334-338). It is an adaptation of Briggs and Shantz's<sup>9</sup> method for the determination of the wilting point of cacti. Briefly, it consists in balancing the potted plant in a horizontal position upon a knife edge placed at the juncture of plant and sealed pot. The plant remains in an erect position excepting for a brief time when it is being balanced by a movable screw. As the plant transpires, water moves from the soil and replaces that lost by the plant, causing the pot side of the system to become lighter and, in consequence, to rise. If the plant does not obtain enough water from the soil to completely replace the loss by transpiration, the plant side of the system rises. By determining the loss in weight from the entire system on a pair of balances, and properly comparing this with the weight which, when placed at the center of pot or center of the aerial part of the plant, restores equilibrium, transpiration and absorption can be separated. A discussion of the limitations of this method and an account of the calculations needed may be found in the paper referred to above (Shreve, E. B. [1915]).

*Results:* Data appear in tables 5 and 6 and in figures 3 and 4. An examination of the graphs for absolute transpiration ( $T$ ) and absolute absorption ( $A$ )

---

<sup>8</sup> For details of the method used in making this correction see: Shreve, Edith B., An investigation of the causes of autonomic movements in succulent plants. *Plant World* **18**: 297-312, 331-343. 1915. Page 333.

<sup>9</sup> Briggs, L. J., and H. L. Shantz, The wilting coefficient for different plants and its direct determination. U. S. Dept. Agric., Bur. Plant Ind., Bull. 230. 1912. Pages 47-51.

TABLE 5

*Simultaneous measurements of transpiration and water absorption by roots. Data expressed as grams per hour. (Experiment 5, part I)*

DATE		TIME	TRANSPIRATION (T)	ABSORPTION (A)	EVAPORATION (E)	TRANSPIRING POWER (T/E)	ABSORBING POWER (A/T)
Plant No. 13	June 28.....	6 p.m.					
	June 29.....	6 a.m. (dk) <sup>a</sup>	0.46	0.02	9.2	0.050	0.04
		6 p.m. (lt)	0.11	0.11	13.5	0.008	1.00
	June 30.....	6 a.m. (dk)	0.38	0.04	7.4	0.051	0.11
		6 p.m. (lt)	0.11	0.24	15.0	0.007	2.18
Plant No. 14	June 28.....	6 p.m.					
	June 29.....	6 a.m. (dk)	0.59	0.42	9.2	0.064	0.71
		6 p.m. (lt)	0.14	0.14	13.5	0.010	1.00
	June 30.....	6 a.m. (dk)	0.37	0.26	7.4	0.050	0.70
		6 p.m. (lt)	0.15	0.22	15.0	0.010	1.40
	July 1.....	6 a.m. (dk)	0.25	0.07	6.0	0.041	0.28

<sup>a</sup> The letters dk denote darkness and lt, light.

TABLE 6

*Simultaneous measurements of transpiration and water absorption by roots (Experiment 5, part II)*

DATE		TIME	TRANSP. (T)	ABSORPT. (A)	EVAP. (E)	TRANSP. POWER (T/E)	ABS. POWER (A/T)
Plant No. 31	June 20	7:50 p.m.					
	June 2	8:13 a.m. (dk) <sup>a</sup>	0.41	0.28	1.36	0.30	0.68
		12:20 p.m. (lt)	0.12	0.52	1.53	0.08	4.33
		3:55 p.m. (lt)	0.20	0.75	2.00	0.10	3.75
		6:45 p.m. (sh)	0.14	0.00+	.....	.....	0.00
Plant No. 30	June 20	8:10 p.m.					
	June 21	7:42 a.m. (dk)	0.32	0.29	1.36	0.24	0.91
		12:4 p.m. (lt)	0.27	0.46	1.53	0.18	1.70
		3:26 p.m. (lt)	0.30	0.15	2.00	0.15	0.50
		8:51 p.m. (lt,dk)	0.15	0.15	2.10	0.07	1.00
	June 22	8:29 a.m. (dk)	0.48	0.27	1.36	0.35	0.56
		12:1 p.m. (lt)	0.30	0.51	1.76	0.17	1.70
		6:40 p.m. (lt)	0.18	0.35	2.71	0.07	1.94
	June 23	7:29 a.m.					
		11:47 a.m. (lt)	0.35	0.38	1.57	0.20	1.00
		3:43 p.m. (lt)	0.20	0.24	2.50	0.08	1.20
Plant No. 34	June 25	6:45 p.m.					
		11:39 p.m. (dk)	0.14	0.22	0.68	0.21	1.57
	June 26	5:20 a.m. (dk)	0.66	0.43	0.46	1.43	0.66
		7:17 a.m. (sh)	0.64	0.86	0.45	1.42	1.33

<sup>a</sup> The letters dk denote darkness; lt, light, and sh, shade.

brings out the fact that, for all the plants, the amount of water absorbed at night is less than the amount transpired, while during the day the amount absorbed is either equal to or greater than the amount transpired. Further, in plants Nos. 13, 30, and 31, the amount taken up at night was actually less than that absorbed during the day. As has been mentioned, the absolute

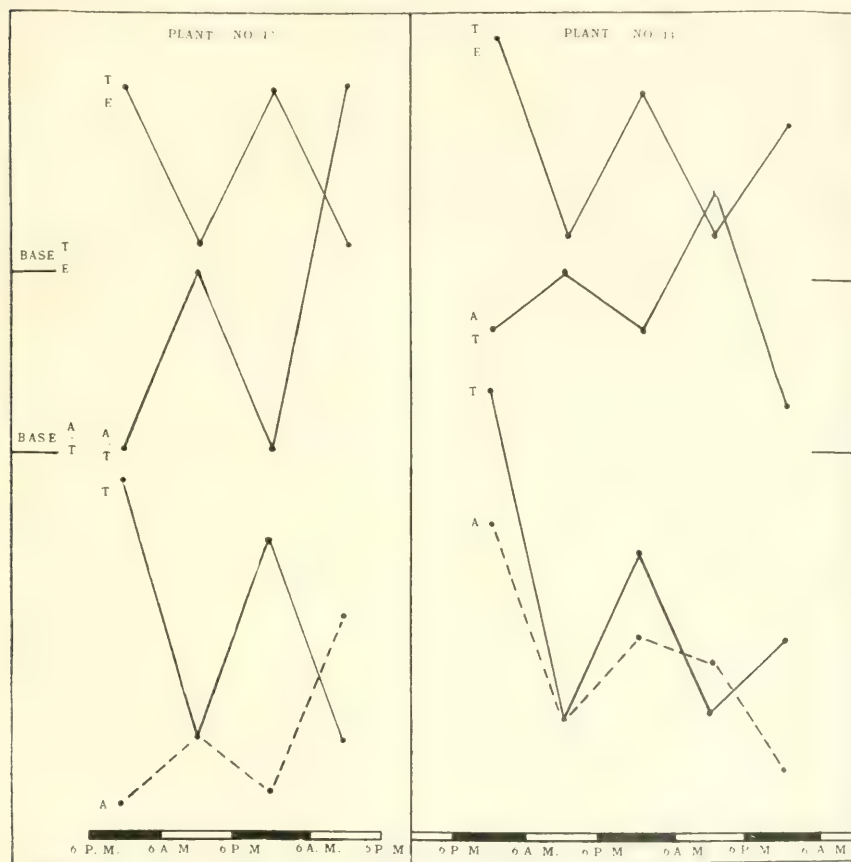


FIG. 3. Absolute absorption, absolute transpiration, secondary absorbing power, and transpiring power of two plants. Readings taken simultaneously on the same plant, in each case. (See experiment 5, pt. I, table 5.)

absorption in this experiment is equivalent to the absorbing power of the plant.

It is thus indicated that the absorbing power has variations which are due to internal causes, just as transpiring power has such variations. Experiment 4 revealed the fact that the cause of the variations in transpiring power cannot be ascribed to changes in absorbing power; let us now consider the converse of this relation.

It is natural to suppose that for every portion of water lost by transpiration there is a corresponding tendency for water to enter at the roots in like amount. This is true no matter what theory is held for the mechanism

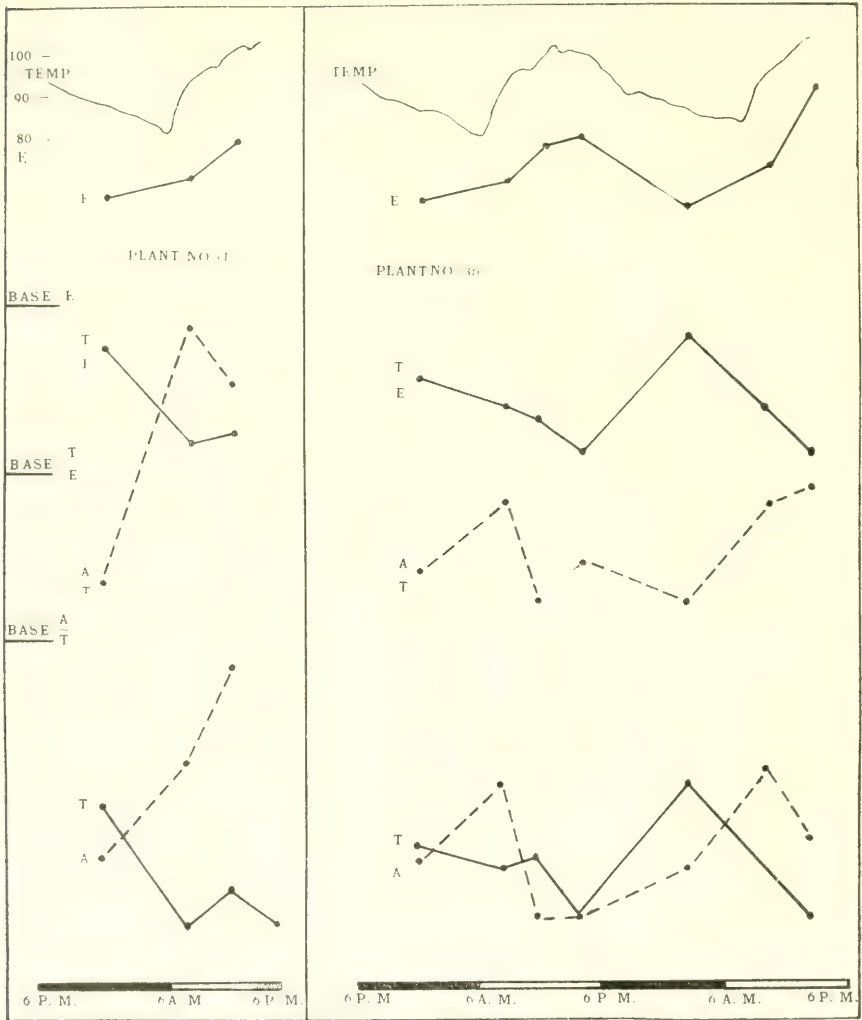


FIG. 4. Absolute absorption, absolute transpiration, secondary absorbing power, and transpiring power of two plants. Readings taken simultaneously on the same plant, in each case. (See experiment 5, pt. II, table 6.)

of water entrance. The outgo of water may be thought of as exercising the same kind of influence on absorption as the desiccating power of the air does on transpiration. Consequently, by following the same line of reasoning

as that used by Livingston in defining  $T/E$  as the transpiring power of the plant, I have obtained  $A/T$  as a tentative measure of that part of the absorbing power which is independent of transpiration. For want of a better name I shall refer to it as the *secondary absorbing power*.

In figures 3 and 4 appear curves resulting from the division of  $A$  by  $T$ . Although the shape and direction of the  $A$ -graphs for the various plants differ among themselves, the  $A/T$ -graphs are very similar. All of the latter show a lower secondary absorbing power at night than during the day. This, it will be noted, is the exact opposite of what is shown for the transpiring power for the same plants.

This experiment has shown that  $A$  (here equivalent to absorbing power) varies definitely from day to night. Let us suppose for a moment that  $T$  (water loss from the plant for the same period) is the sole factor in governing the variations in  $A$ . In this case the graphs of  $A$  and  $T$  would have the same shape, the quotient  $A/T$  would be a constant, and,—if the relation were a simple, direct one,—its graph would be a horizontal line. Figures 5 and 6 show that the graphs of  $A$  and  $T$  follow opposite directions. Therefore  $T$  is not a factor which acts in the same direction as the final result. The conclusion is, then, that some other factor or group of factors is of major importance in controlling the variations in  $A$ . The controlling factor cannot be a constant and must vary in a direction always opposite to that of the variations in  $T$ .

### INTERRELATIONS OF $T/E$ , $A/T$ , AND WATER-HOLDING CAPACITY OF TISSUES

It must be remembered that there are internal changes in the plant that cause variations in the transpiring power. Now, it is possible that the same change in internal conditions which causes an increase in the absorbing power may at the same time cause a decrease in transpiring power, since many non-stomatal changes that would cause a decrease in transpiring power would theoretically cause an increase in absorbing power. For example, if there occurred an increase in the concentration of cell sap, while all other conditions within and without the cell remained the same, there would result (1) an increased rate of water entrance to the cell, and (2) a decrease in the rate of water given off to the cell walls and thus made available for evaporation. To give another example, if there occurred periodically an increase in the imbibitional capacity of the hydrophile colloids of the tissues there would result (1) an increase in the rate of water entrance and (2) an increase in the water-holding capacity of the tissue, and consequently a stronger resistance to the conduction of water to the evaporating surfaces. A decrease in imbibitional capacity would, on the other hand, lessen the absorbing capacity and lower the resistance to water movement, and hence

an increase in transpiring power would result, while at the same time less water would enter the cell.

It is well known that the chemical constitution of living plant material alters with changes in temperature and light intensity, and there are theoretical reasons for supposing that the increase and decrease of the various compounds may bring about variations in the water-holding power of the tissue. So it seems desirable to continue the investigation into the causes of the variations of  $T/E$  and  $A/T$ , by a study of the influence of light intensity and temperature on them.

When the graphs of experiment 5 are examined it is seen that they are related in such a way that one is approximately the mirror image of the other. This experiment shows the relation for only one set of light and temperature conditions. A test for the theory advanced in the preceding paragraph should logically begin with the effect of other conditions on the relation itself, and such a beginning is made in the next experiment.

### Experiment 6

*Object:* The determination of the march of transpiring power under conditions of direct sunlight and high temperatures, followed by darkness and low temperatures.

*Material:* Plants Nos. 2, 3, 9, 14, and 15, the histories of all of which have been given above.

TABLE 7

*Absolute and relative transpiration with reference to light and darkness conditions. Data expressed as grams per hour. (Experiment 6, figure 5)*

DATE	TIME	TRANSPARATION (T)					TRANSPIRING POWER ( $T/E$ )					EVAPORATION ( $E$ )
		No. 2	No. 3	No. 9	No. 14	No. 15	No. 2	No. 3	No. 9	No. 14	No. 15	
Nov. 1	6:30 p.m.											
	12:5 a.m. (dk) <sup>a</sup>	0.039	0.170	0.110	0.095	0.043	0.085	0.369	0.239	0.206	0.093	0.46
Nov. 2.	7:20 a.m. (dk)	0.168	0.190	0.241	0.100	0.092	0.369	0.422	0.536	0.222	0.204	0.45
	11:59 a.m. (lt)	0.068	0.072	0.088	0.054	0.064	0.094	0.100	0.122	0.075	0.089	0.72
	3:17 p.m. (lt)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.80
	5:41 p.m. (lt)	0.000	0.033	0.021	0.000	0.008	0.000	0.081	0.051	0.000	0.019	0.41
Nov. 3..	9:8 p.m. (dk)	0.043	0.006	0.043	0.000	0.014	0.105	0.015	0.105	0.000	0.034	0.41
	12:5 a.m. (dk)	0.112	0.119	0.136	0.044	0.068	0.248	0.265	0.302	0.098	0.151	0.45
	3:37 a.m. (dk)	0.162	0.170	0.127	0.112	0.098	0.317	0.333	0.149	0.219	0.192	0.51
	12:12 p.m. (dk)	0.111	0.160	0.041	0.055	0.072	0.222	0.320	0.082	0.110	0.144	0.50
Nov. 4..	3:30 p.m. (lt)	0.000	0.184	0.000	0.000	0.004	0.000	0.409	0.000	0.000	0.009	0.45
	6:12 p.m. (lt)	0.067	0.039	0.119	0.022	0.045	0.067	0.039	0.119	0.022	0.045	1.00
	9:2 a.m. (dk)	0.097	0.141	0.093	0.045	.....	0.220	0.320	0.211	0.102	.....	0.44
Nov. 4..	12:5 p.m. (lt)	0.016	0.017	0.098	0.050	0.069	0.018	0.019	0.110	0.056	0.077	0.89

<sup>a</sup> The letters dk. denote darkness and lt., light.

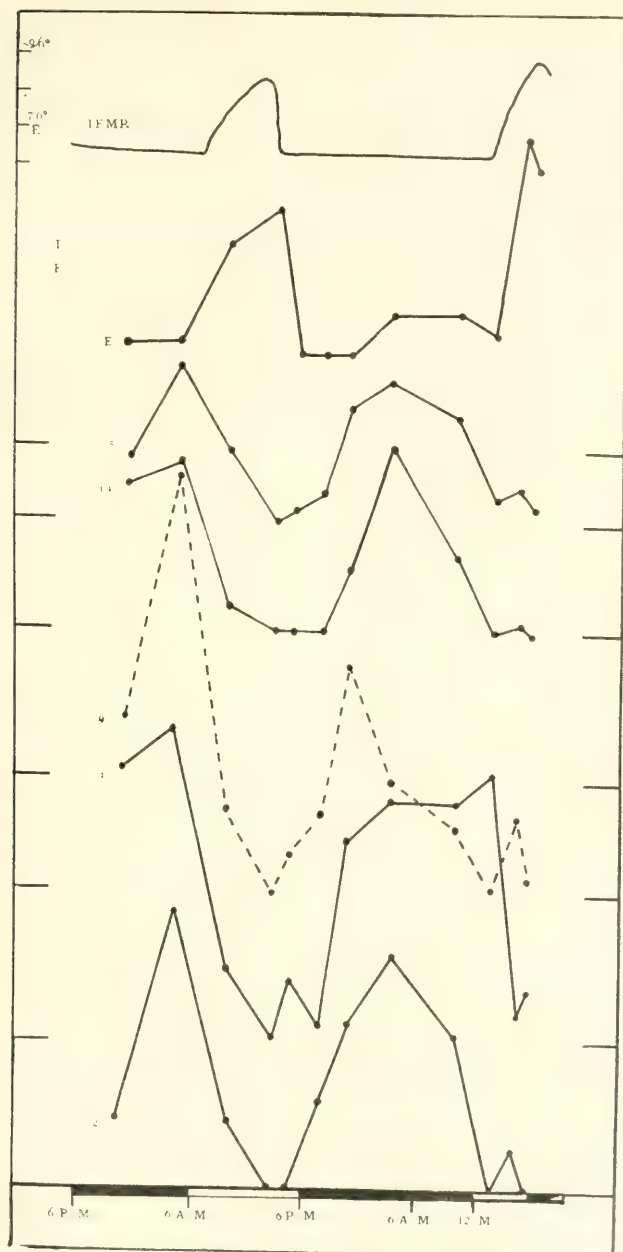


FIG. 5. Daily march of transpiring power of five plants under natural environmental conditions. (See experiment 6, table 7.)

*Method:* The plants were sealed and treated, in all respects, as in experiment 3. The tissues were slightly below their maximum turgidity but they were not desiccated. During the day the plants were in the sunlight in the green-house and at night in a dark room. On the second day all plants were left until noon in the dark room, where the temperature remained below 18.5°C. (65°F.)

*Results:* Data appear in table 7 and figure 5. So long as the conditions of strong light and high temperature prevail, the readings, taken here at more frequent intervals, agree with those in previous experiments and show, in addition, the following: (1) There is more variation here between individual plants; (2) the time of maximum transpiring power varies with different plants but generally occurs after midnight; (3) the transpiring power continues to decrease during the greater part of the day, but frequently begins to increase in the afternoon; (4) it increases during the greater part of the night but usually begins to decrease from the maximum before daylight; (5) all of the individual values for the night are higher than any one obtained for the daytime; (6) on the second day, when the plants were retained in the dark room at a temperature of about 18°C., (65°F.) the transpiring power continued to remain at the high night value as long as the unusual conditions prevailed.

#### *Experiment 7*

*Object:* An investigation of the influence of a constant temperature of 32°C. (90°F.) throughout the night upon transpiring power and water intake.

*Material:* Plants Nos. 2 and 3, which were green-house grown, and No. 20, an individual which had spent its entire life in the open.

*Method:* Plants were sealed in aluminum shells, as in former experiments, and weighed at intervals. For the first 24 hours of the experiment the plants were left in the green-house during the day and placed in a cool, dark room at night. At the close of the first day, when the plants were found to be transpiring as in former experiments (i.e., showing a greater value of  $T/E$  for night than for day), they were placed in a glass cage of 15 cu. ft. capacity, where the temperature was automatically held at 32°C. (90°F.). Hygrograph records showed that the humidity of the cage varied not over 5 per cent. from that of the air in the green-house or dark room at corresponding times. The sunlight entered the cage during the daylight hours but after 2 o'clock it rendered the temperature of the cage too hot even with the door wide open, and consequently, the plants were transferred to the open after that hour. On the second night plant No. 3 was returned to the cage and kept at 32°C. (90°F.) again for the night, but Nos. 2 and 20 were placed in the dark room where the temperature varied from 18.4° to 15.6°C. (65° to 60°F.). From the morning of the third day until the end of the experiment all of the plants had the same treatment.

*Results:* Data appear in tables 8 and 9 and in figures 6 and 7. All three plants show that when the night temperature is held at 32°C. (90°F.) the

TABLE 8

Absolute and relative transpiration for three plants under conditions of high nocturnal temperature. Data expressed as grams per hour. (Experiment 7, figs. 6 and 7)

DATE	TIME	ABSOLUTE TRANSPIRATION (T)				TRANSPIRING POWER ( $T/E$ )				EVAPORATION	
		No. 2	No. 3	No. 20	No. 2	No. 3	No. 20	No. 20	No. 20	E <sub>1</sub> a	E <sub>2</sub> b
Apr. 8 . . .	5:58 p.m.										
	7:40 a.m. (dk) <sup>d</sup>	0.28(D) <sup>c</sup>	0.14(D)	0.05(D)	0.56(D)	0.28(D)	0.10(D)			0.50	0.50
	12:43 p.m. (lt)	0.04(G)	0.02(G)	0.04(G)	0.05(G)	0.03(G)	0.05(G)			0.79	0.79
Apr. 9 . . .	7:07 p.m. (lt)	0.03(G)	0.03(G)	0.02(G)	0.04(G)	0.04(G)	0.02(G)			0.85	0.85
	11:48 p.m. (dk)	0.04(C)	0.04(C)	0.03(C)	0.03(C)	0.03(C)	0.02(C)			1.25	1.25
	5:25 a.m. (dk)	0.04(C)	0.02(C)	0.03(C)	0.03(C)	0.02(C)	0.02(C)			1.25	1.25
Apr. 10 . . .	7:24 a.m. (sh)	0.05(C)	0.07(C)	0.05(C)	0.04(C)	0.05(C)	0.04(C)			1.31	1.31
	10:44 a.m. (lt)	0.27(C)	0.11(C)	0.09(C)	0.12(C)	0.05(C)	0.04(C)			2.28	2.28
	12: 4 p.m. (lt)			0.09(D)			0.03(D)			3.52	
	7:22 p.m.										
	9:32 p.m. (dk)	0.19(D)	0.08(C)	0.07(D)	0.24(D)	0.06(D)	0.09(D)			1.36	
	11:48 p.m. (dk)	0.22(D)	0.04(C)	0.13(D)	0.33(D)	0.03(C)	0.20(D)			1.37	
	1:49 a.m. (dk)	0.40(D)	0.07(C)	0.22(D)	0.60(D)	0.05(C)	0.31(D)			1.42	
	6:2 a.m. (dk)	0.47(D)	0.08(C)	0.20(D)	0.75(D)	0.06(C)	0.32(D)			1.42	
	8:30 a.m. (sh)	0.22(C)	0.04(C)	0.17(C)	0.21(C)	0.03(C)	0.16(C)			1.54	
Apr. 11 . . .	10:18 a.m. (lt)	0.03(C)	0.07(C)	0.10(C)	0.01(C)	0.03(C)	0.04(C)			2.28	
	12:36 p.m. (lt)	0.13(C)	0.28(C)	0.05(C)	0.05(C)	0.10(C)	0.02(C)			2.80	
	2:27 p.m. (lt)			0.07(C)			0.02(C)			3.81	
	5:12 p.m. (lt)			0.06(O)			0.01(O)			4.92	
	7:27 p.m. (lt)			0.24(O)			0.07(O)			3.61	
	9:26 p.m. (dk)	0.25(D)	0.17(D)	0.13(D)	0.26(D)	0.18(D)	0.13(D)			0.96	
Apr. 12 . . .	11:25 p.m. (dk)	0.27(D)	0.22(D)	0.10(D)	0.34(D)	0.28(D)	0.13(D)			0.78	
	7:29 a.m. (dk)	0.55(D)		0.15(D)	0.74(D)		0.20(D)			0.74	
	10:4 a.m. (lt)	0.27(G)		0.14(G)	0.33(G)		0.17(G)			0.83	
	11:54 a.m. (lt)	0.05(G)		0.05(G)	0.04(G)		0.04(G)			1.14	

<sup>a</sup> E<sub>1</sub> from atmometer with same environment as plants Nos. 2 and 20.

<sup>b</sup> E<sub>2</sub> from atmometer with same environment as plant No. 3.

<sup>c</sup> Letters in parentheses at the right of numbers indicate the environment of the plants during the period covered by the reading, the abbreviations being as follows:

D, dark room, 15.5-21°C. (60-70°F.);

G, greenhouse, 15.8-32.2°C. (60-90°F.);

C, constant temperature cage, 32.2°C. (90°F.);

O, open, on laboratory porch.

<sup>d</sup> The letters dk denote darkness; lt, light; and sh, shade.

usual high transpiring power for the night does not appear but, instead, the low rate of the day continues, with very little change.

In a previous paper [1915] I have shown that movements occur in *Opuntia versicolor* which are caused by changes in the relation of absolute transpiration to absolute absorption at the roots. An upward movement occurs when more water is absorbed than is lost and a downward movement when more is lost than absorbed. Consequently, a measurement of the movements

TABLE 9

*Movements of joints, occurring simultaneously with the transpiration measurements given in table 8. Data expressed as centimeters. (Experiment 7)*

DATE	TIME	No. 3	No. 2.		
			Joint A	Joint B	Joint C
Apr. 8 .....	6:30 p.m.				
	7:40 a.m. (dk) <sup>a</sup>	-3.8(D) <sup>a</sup>	-0.9(D)	-0.2(D)	-0.2(D)
	12:45 p.m. (lt)	+4.3(G)	+1.8(G)	+0.7(G)	+0.5(G)
Apr. 9 .....	7:10 p.m. (lt)	+1.3(G)	-0.1(G)	0.0(G)	+0.1(G)
	11:50 p.m. (dk)	+0.4(C)	0.0(C)	0.0(C)	+0.1(C)
	5:30 a.m. (dk)	+0.4(C)	-0.3(C)	-0.1(C)	0.0(C)
	7:25 a.m. (sh)	0.0(C)	-0.3(C)	-0.1(C)	0.0(C)
	10:45 a.m. (lt)	-0.3(C)	-1.0(C)	+0.1(C)	-0.3(C)
Apr. 10 .....	11:45 a.m. (lt)	-1.5(C)	-1.0(C)	-1.0(C)	-0.1(C)
	1:00 p.m. (lt)	-4.3(O)	-1.9(O)	-0.1(O)	-0.4(O)
	7:00 p.m.				
	9:40 p.m. (dk)	+1.7(C)	0.0(D)	0.0(D)	-0.2(D)
	12 night (dk)	+2.1(C)	-0.1(D)	+0.1(D)	+0.1(D)
	2:00 a.m. (dk)	+0.5(C)	-0.1(D)	-0.2(D)	-0.1(D)
	5:30 a.m. (dk)	+0.8(C)	-0.8(D)	-0.4(D)	-0.5(D)
	8:30 a.m. (lt)	+1.6(C)	-1.1(C)	+0.2(C)	0.0(C)
	10:30 a.m. (lt)	-1.3(C)	+0.9(C)	+0.5(C)	+0.6(C)
Apr. 11 .....	12:30 p.m. (lt)	-1.5(C)	+2.6(C)	+0.7(C)	+0.3(C)
	2:30 p.m. (lt)	-4.7(C)	+0.5(C)	0.0(C)	-0.7(C)
	7:30 p.m. (lt, sh)	+0.1(O)	-0.5(O)	-0.3(O)	-0.3(O)
	9:30 p.m. (dk)	-0.4(D)	-0.1(D)	0.0(D)	+0.1(D)
	11:30 p.m. (dk)	-0.8(D)	-0.1(D)	+0.2(D)	+0.1(D)
	7:30 a.m. (dk)	-3.0(D)	-3.3(G)	-1.4(G)	-0.8(G)
Apr. 12 .....	10:00 a.m. (lt)		+1.7(G)	+0.4(G)	+0.7(G)
	12 noon (lt)		+0.6(G)	+0.5(G)	0.0(G)

<sup>a</sup> Letters in parentheses have same meaning as in table 8.

ought to indicate changes in the net water-content of the tissues. In table 9 are given the movements of one branch of plant No. 3 and three branches of plant No. 2. The plus sign indicates an upward movement and the minus a downward one. Or, to interpret these movements in terms of the water-content of the tissues, a plus sign shows an increase in water-content for the

period considered over the previous period, and a minus sign indicates a decrease from the period immediately preceding.

The conclusion to be drawn is that when a high night temperature prevails the relation between intake and outgo is the same as that which exists during the daytime. Since the first part of this experiment has shown that  $T/E$

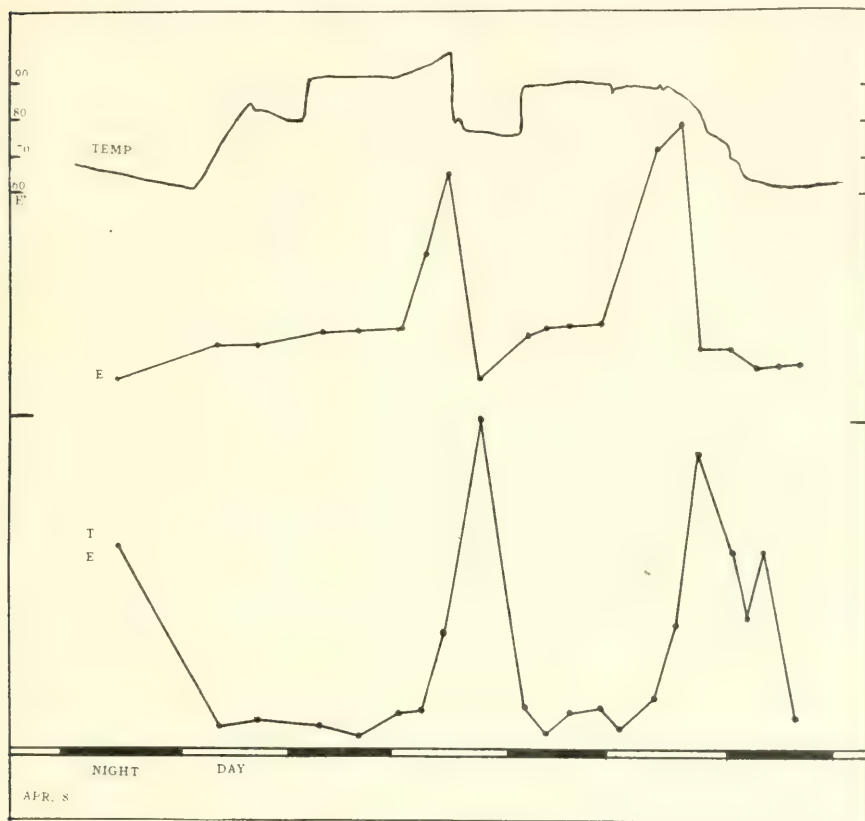


FIG. 6. Transpiring power of plant No. 3. Two nights, with temperature above  $32.2^{\circ}\text{C}$ . ( $90^{\circ}\text{F}$ .), typical conditions for remaining time. (See experiment 7, table 8.)

follows the daytime behavior at night, with a temperature of  $32^{\circ}\text{C}$ . ( $90^{\circ}\text{F}$ .), it may be inferred that  $A/T$  probably also behaves as it does in the day.

In the next experiment a direct method of measuring absorption is attempted, the environmental conditions being similar to those which existed in the last experiment.

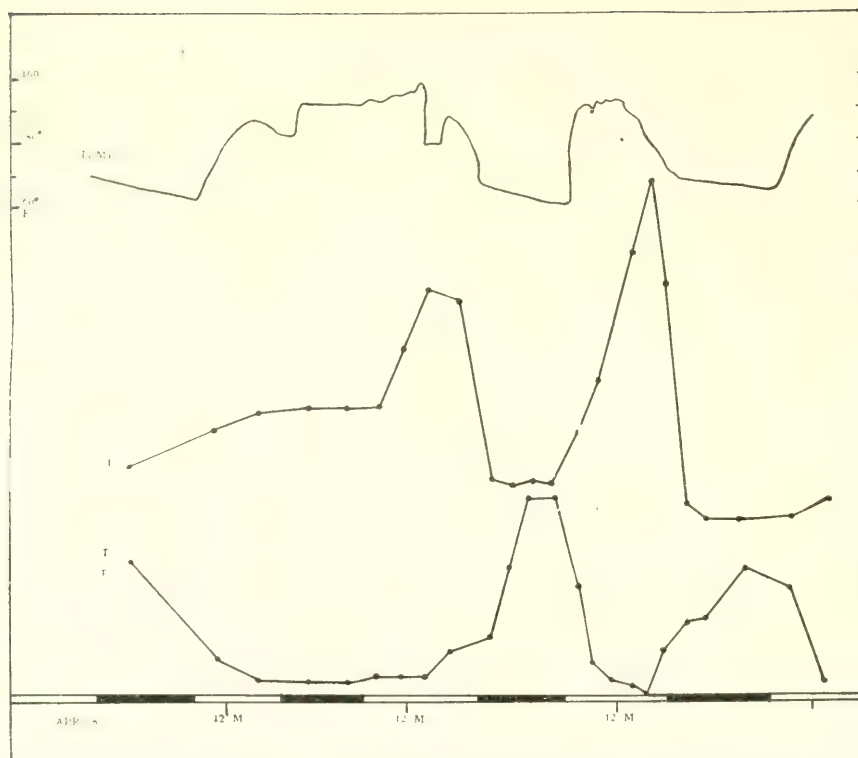


FIG. 7. Transpiring power of plant No. 20. One night with temperature above  $32.2^{\circ}\text{C}$ . ( $90^{\circ}\text{F}$ .), typical conditions for remaining time. (See experiment 7, table 8.)

### Experiment 8

*Object:* The determination of the influence of certain controlled temperature conditions on the transpiring power of the plant and on the secondary absorbing power of its roots.

*Material:* Plants Nos. 32 and 33. They were about 5 years old at the time of the experiment. About 8 weeks before they were used they were transplanted from the open and placed in well-drained, galvanized iron cylinders, where they soon became well established.

*Method:* The plants were sealed and treated in all respects as were those in experiment 5, part II. High night temperatures were obtained in a laboratory dark room without the use of artificial heat. A refrigerated glass cage furnished the low temperatures.

*Results:* Data are given in table 10 and figure 8. It will be seen that hot nights with temperature above  $32^{\circ}\text{C}$ . ( $90^{\circ}\text{F}$ .) give very different values of  $A/T$  from those given by cool nights with temperature maxima below  $21^{\circ}\text{C}$ .

(70°F.). In both plants  $A/T$  is greater than unity on the hot nights and less than unity on cool ones. A hot night affects the value of  $A/T$  for the following day, so that this value falls below unity, although the day is one of typical temperature and sunshine. The next following night (15.6°C. or 60°F.) shows this value somewhat below unity, and, during the day following that, the value at last becomes greater than unity again. By the third day the plants have returned to their normal variation in the value of  $A/T$ .

TABLE 10

*Simultaneous measurements of transpiration and root absorption under various temperature conditions. Data expressed as grams per hour. (Experiment 8, figure 8)*

DATE	TIME	ABS. TRANSP. ( $T$ )	ABSORPT. ( $A$ )	EVAP. ( $E$ )	TRANSP. POWER ( $T/E$ )	ABS. POWER ( $A/T$ )	TEMPERATURE
							deg. C.
Plant No. 32	June 23.....						
	7:56 p.m.						
	8:50 a.m. (dk) <sup>a</sup>	0.28	0.67	1.34	0.21	2.39	35-32.7
	12:35 p.m. (lt)	1.41	1.01	1.57	0.90	0.72	36.7-39.5
	June 24.....						
	3:20 p.m. (lt)	1.23	0.65	2.61	0.47	0.53	39.5-40
	7:27 p.m. (lt,sh)	1.31	1.07	2.53	0.51	0.82	40-34.5
	11:29 p.m. (dk)	0.12	0.12	0.93	0.13	1.00	22.2-18.9
	5:34 a.m. (dk)	0.75	0.33	0.44	1.70	0.44	18.9-16.7
	9:39 a.m. (lt)	0.78	0.71	1.26	0.61	0.91	28.9-35.5
	June 25.....						
	5:32 p.m. (lt)	1.30	1.22	2.60	0.50	0.94	35.5-38.9-37.8
	6:42 p.m. (sh)	0.34	.....	1.52	0.22	.....	.....
	11:34 p.m. (dk)	0.74	0.58	0.68	1.09	0.78	21.2-18.4
	June 26.....						
	5:12 a.m. (dk)	1.29	0.87	0.46	2.80	0.67	18.4-17.2
	7:7 a.m. (sh)	1.31	1.31	0.45	2.94	1.00	.....
Plant No. 33	June 23.....						
	7:40 p.m.						
	9:6 a.m. (dk)	0.45	0.48	1.34	0.34	1.07	35-32.7
	1:21 p.m. (lt)	0.09	0.09	1.57	0.06	1.00	36.7-40
	June 24.....						
	7:19 p.m. (lt,sh)	0.45	0.08	2.48	0.18	0.18	40-34.5
	11:20 p.m. (dk)	0.10	0.10	0.93	0.11	1.00	22.2-18.9
	5:28 a.m. (dk)	0.15	0.13	0.44	0.34	0.87	18.9-16.7
June 25	9:31 a.m. (lt)	0.44	0.52	1.26	0.35	1.18	28.9-35.5
	1:4 p.m. (lt)	0.42	0.30	2.70	0.15	0.71	35.5-39.5

<sup>a</sup> The letters dk denote darkness; lt, light, and sh, shade.

The last two experiments show that a high night temperature upsets the regular march of both  $T/E$  and  $A/T$ . Evidence of this fact may also be found in the paper referred to above, for the regular sequence of short period movements of this cactus (i.e., *up* in the daytime and *down* at night) was interrupted when the plants were subjected to: (1) constant temperature of 35°C. (95°F.) with an alternation of diffuse light and darkness at 12-hour intervals, (2) constant temperature of 18.3°C. (65°F.) with constant darkness, (4) constant temperature of 35°C. (95°F.) with an alternation of sun-

light and darkness at 12-hour intervals, (5) constant darkness with an alternation, at 12-hour periods, of constant temperatures of 35°C. (95°F.) and 18.3°C. (65°F.) It was found also that, when typical conditions were again allowed to affect the plants, normal movements were resumed only after

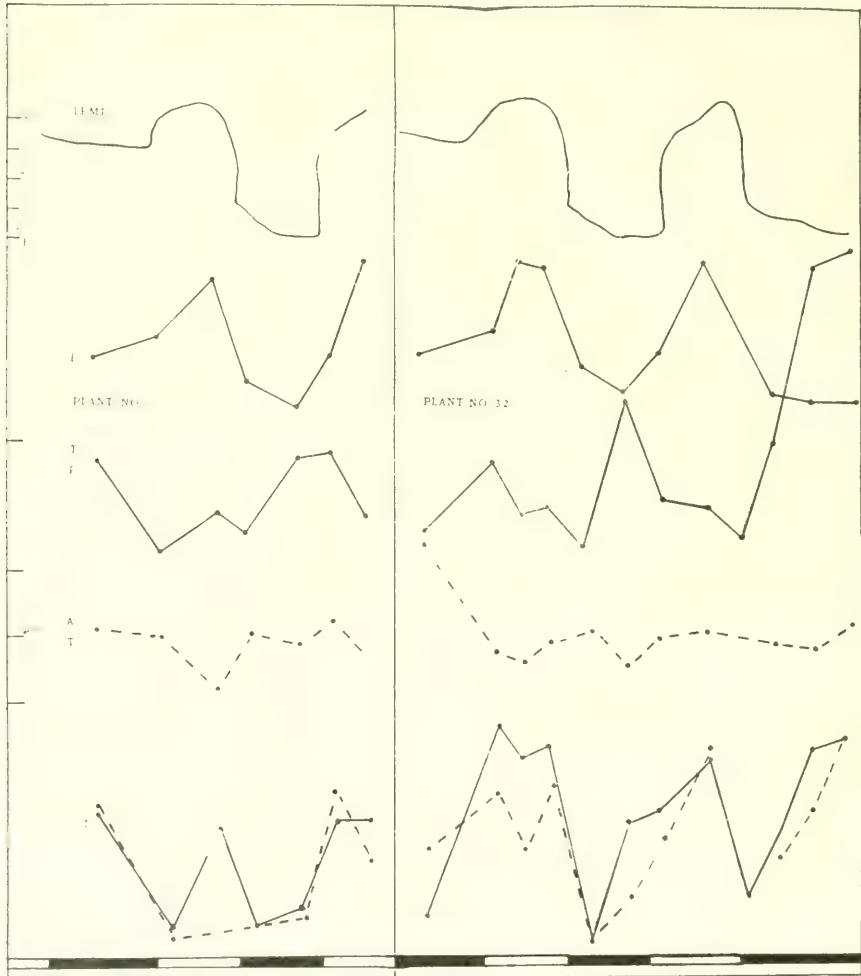


FIG. 8. Absolute absorption, absolute transpiration, secondary absorbing power, and transpiring power of two plants. Readings taken simultaneously on the same plant, in each case. First night temperature above 32.2°C. (90°F.), conditions typical for rest of time. (See experiment 8, table 10.)

the plants had experienced either 12 or 24 hours of typical conditions. Since facts were presented in the same paper proving that these movements are due to changes in water-content that were, in turn, due to changes in the

differences between  $A$  and  $T$ , it is evident that these interruptions to the normal environmental conditions upset the normal sequence of the values of either  $A$  or  $T$  or both. The above considerations, together with the facts obtained from experiments 6 and 7, strengthen the evidence that changes of light and temperature affect  $T/E$  and  $A/T$ . That the effects of light and temperature are indirect must be deduced from various cases where a return to a given set of conditions did not bring about an immediate return of the previous behavior. If the influence were direct we should expect a certain combination of light and temperature always to bring about the same result, which is surely not the case as shown by the experimental data.

Livingston [1907] found that 16 per cent. of his cactus plants showed abnormal behavior with reference to the daily variations in  $T/E$ , and suggested that this might have been due to pathological conditions. I have found from the experiments here reported, and from as many more, that  $T/E$  (dk)  $>$   $T/E$  (lt) without exception, (1) if the tissues are turgid, (2) if the days are clear with bright sunshine and with a maximum temperature above 21.2°C. (70°F.) and (3) if the nights have a maximum temperature below 29.4°C. (85°F.). I have found also that a change of any one of the above conditions will make  $T/E$  (dk) either equal to or less than  $T/E$  (lt). For convenience, I shall hereafter call typical those conditions which bring about the relation  $T/E$  (dk)  $>$   $T/E$  (lt), and atypical, those which cause a different relation of these ratios. That is, typical conditions are those under which the transpiring power of this plant is greater by night than by day.

As was mentioned on a previous page, the succulent type of plant retains within its tissues a considerable proportion of the water it consumes in a given year. This water is held by the so-called storage tissue, in such a way that there is within each joint a more extensive continuous connection between wet cells than exists in non-succulents. In fact, at all points, excepting at the connections between joints (where only vascular bundles pass), there is apparently as close connection between wet cells as there is in the mesophyll of a thin leaf. It has already been shown that there is no direct relation between the transpiration rate and the rate of water intake at the roots, and so it seems reasonable to suppose that the water-storage tissue exerts some influence upon transpiration and absorption. This tissue may be supposed to have an absorbing or water-holding capacity of its own, due, perhaps, to the presence of differential diffusion phenomena and also to imbibitional forces. Any changes which could be discovered in the absorbing capacity of this tissue might throw light on the problem in hand. This matter was investigated in experiment 9.

*Experiment 9*

*Object:* Measurement of the absorbing and water-holding capacity of internal tissue.

*Material:* Year-old joints from plants F and H, growing in the open; half-year-old joints from plant E, also growing in the open; 1 year and 2 year old joints from a potted plant, No. 4, which had formed all of its branches in the open, with the exception those one year old, these having been formed in the green-house; joints more than two years old from a potted plant, No. 9, which had spent its entire life in the green-house; half-year-old joints from a potted plant, No. 20, which had also spent its entire life in the green-house.

*Method:* From the stems of all the plants, excepting F, small cylinders of uniform shape and size were cut in a longitudinal direction, by means of a cork borer. Both the epidermis and the ring of vascular tissue were excluded. At each cross-section, usually from 3 to 4 cylinders were obtained from the region inside the vascular ring and from 5 to 6 outside the ring. The material from the two regions was treated separately on July 14-15, the results being shown in table 11, A. In November the two regions were treated together (table 11, B and C). In the case of plant F., larger cylinders were cut cross-wise of the stem, thus including all of the tissues with the exception of the epidermis, which was removed with forceps. On July 14-15 the samples were taken first at 5 p.m., and again on the following morning, at 5 a.m. On Nov. 3-5 they were taken first in the morning and next on the evening of the same day. The material from each joint was treated separately. In some instances the material from a given plant came from two or three joints, and in such cases the different sets of cylinders were kept separate and designated "lot A, lot B," etc. (See table 11, C.)

As soon as the cylinders were cut they were gently rolled on filter paper until the surfaces appeared dull, then quickly put into covered weighing bottles and weighed. They were next immediately placed in distilled water. Whenever they were reweighed they were taken from the water and dried on the surface as above. Tests for the error caused by this method of drying were made by repeated drying and rewetting of the same sample; the error was found to be between 0.001 and 0.002 g. for 10 cylinders taken together. Since no figures smaller than 0.01 g. were retained in the final values, this error is considered as negligible. Weighings were made at half-hour intervals for 1.5 hours, and after that, every 15 minutes. After the maximum absorption had been reached the weight always began to decrease and so the 15-minute intervals were used, to detect the maximum. While the tissue was absorbing water it was kept at a nearly constant temperature of 21°C. (70°F.). The samples which were taken at 5 p.m. were kept in diffuse light in the laboratory until dusk and were then placed under the light of a 150-candle-power electric light; those samples which were obtained at 5 a.m.

TABLE 11

Water-content and water-holding capacity of internal tissue. Data are given in terms of grams per gram of dry weight. (Experiment 9)

A. Tissue from center and periphery of joint, taken separately, at 5 a.m. and 5 p.m., July 14-16

PLANT	AGE OF JOINT		5 p.m.		5 a.m.			
			Tissue from		Tissue from			
			center of joint	periphery of joint	center of joint		periphery of joint	
					Lot A	Lot B	Lot A	Lot B
	<i>years</i>							
H	1	Original water-content.....	10.7	7.2	10.0	10.0	7.1	7.1
		Water absorbed.....	6.2	0.8	3.7	3.4	0.0	0.3
		Maximum water-content.....	16.9	8.0	13.7	13.4	7.1	7.4
4	1	Original water-content.....	16.2	19.7	15.4	.....	19.4	.....
		Water absorbed.....	7.6	6.1	4.8	.....	3.3	.....
		Maximum water-content.....	23.8	25.8	20.2	.....	22.7	.....
4	2	Original water-content.....	18.2	15.1	.....	.....	10.9	.....
		Water absorbed.....	5.3	4.6	.....	.....	2.8	.....
		Maximum water-content.....	23.5	19.7	.....	.....	13.7	.....
9	2+	Original water-content.....	17.6	14.7	.....	.....	11.9	.....
		Water absorbed.....	1.8	1.0	.....	.....	0.0	.....
		Maximum water-content.....	19.4	15.7	.....	.....	11.9	.....

B. Tissue from center and periphery of joint, taken together, at 5 a.m. and 5 p.m., Nov. 3-5

PLANT	AGE OF JOINT		TISSUE FROM CENTER AND PERIPHERY TOGETHER				
			5 a.m.			5 p.m.	
			Lot A	Lot B	Lot C	Lot A	Lot B
	<i>months</i>						
20	6	Original water-content.....	10.8	13.8	.....	16.0	18.1
		Water absorbed.....	2.1	2.9	.....	3.1	2.3
		Maximum water-content.....	12.9	16.7	.....	19.1	20.4
E	6	Original water-content.....	10.2	9.1	9.8	12.8	13.4
		Water absorbed.....	2.0	2.0	2.8	3.2	3.1
		Maximum water-content.....	12.2	11.1	12.6	16.0	16.5

C. Tissue from center and periphery of joint, taken together, at various hours, Nov. 3-5

PLANT	AGE OF JOINT		1:30 p.m.	6:00 p.m.	11:30 p.m.	6:00 a.m.	11:00 a.m.	3:00 p.m.
	<i>months</i>							
F	6	Original water-content.....	11.49	10.82	9.55	8.48	11.27	9.64
		Water absorbed.....	1.74	1.64	1.75	0.98	1.72	1.75
		Maximum water-content.....	13.23	12.46	11.30	9.30	12.99	11.39

were kept in darkness at about  $21^{\circ}\text{C}$ . ( $70^{\circ}\text{F}$ .) except during the brief time necessary for the weighings. The above conditions were chosen as ones which would probably give as near a mean as possible, between the errors caused by differences of absorption due to temperature and those due to chemical changes within the tissue, due to temperature and light.<sup>10</sup> After the maximum absorption had been determined the dry weight was obtained, by drying the cylinders to constant weight, in an electric oven at  $100^{\circ}\text{C}$ .

The amount of water the tissue is capable of absorbing depends partly upon the amount which it already holds, and since the water-content of different joints varies, a comparison of their absolute absorbing capacity probably means very little as applied to the immediate problem. More significant data can be obtained by comparing the maximum water-content, i.e., the water-holding capacity, for the various tissues. Although the dry weight doubtless changes from time to time, it surely does not change to so great an extent in this plant as does the amount of water present; therefore the calculations have all been reduced to a dry-weight basis.

*Results:* The data given in table 11 show three main facts: (1) there is an agreement between values when a comparison is made of duplicate samples taken at the same time of day from different joints of the same age and from the same plant; (2) there is a disagreement when samples of different ages are taken from the same plant at the same time; (3) when material of the same age from the same plant is compared for different times of day, the maximum water-holding capacity for 6 p.m. is always greater than for 6 a.m.

In the case of plant F, where determinations were made at more frequent intervals, the following additional facts appear: (1) The maximum water-holding capacity begins decreasing sometime between noon and 6 p.m. and continues to decrease until 6 a.m., the greatest decrease occurring between midnight and 6 a.m.; (2) the absolute absorbing capacity remains practically the same from 1:30 p.m. until midnight, decreases from midnight to 6 a.m., and returns to its former value before noon of the next day.

Many other tests of the water-absorbing and water-holding capacity of this tissue were made, the results of which do not appear in the table. Of a hundred tests made, 81 agreed with the conclusions stated above, the remaining 19 showing no measurable change between day and night. Of the 19 exceptional cases, 14 were taken on cloudy days. When the variations in individual joints are taken into account, it seems surprising that there was not a larger number of disagreeing tests.

A comparison of the variations in the water-holding capacity of the tissues, as shown in the last experiment, with the variations in  $T/E$  and  $A/T$  from former experiments, shows that the water-holding capacity varies directly with  $A/T$  and indirectly with  $T/E$ . This relation holds not only for the

---

<sup>10</sup> For a better method of treating the absorbing tissue see experiment 10.

day and night determinations but throughout the 24 hours. It holds not only under typical conditions but also when the sequence of changes in  $T/E$  and  $A/T$  is interrupted by changes in temperature or by desiccation. The truth of the last statement is shown by the following two experiments.

### *Experiment 10*

*Object:* The comparison of the variations of the water-holding capacity of tissues from plants which had been under typical conditions with these variations in tissue from plants which had been under atypical conditions, i.e., in this instance, kept at 35°C. (95°F.) throughout the night.

*Material:* Potted plants from 2 to 10 years old which had been in the green-house for about 6 months. Many of them are the same plants which were used in experiments 1-7. The greatest care was taken to compare only branches of the same age and those that had the same history.

*Method:* For a given set of readings two plants were selected which were as nearly alike as possible, in size and age of their branches. Each plant had, among other joints, two which arose from the end of the same parent joint. Both plants were left in the green-house during the day, and at 5 p.m. one of them was placed in a constant temperature cage, while the other was left in the green-house or placed in the open, for the night hours. At the time of the determinations, one of the two similar joints just mentioned was removed from each plant, by breaking at the point of natural segmentation. Cylinders were cut longitudinally with as large a cork borer as the diameter of the joint would allow, practically all of the tissue excepting the epidermis being included. The same cork borer was always used on the same plant but different sizes were used on different plants, in order that practically all of the tissue might be included in every sample. All cylinders were 2 cm. long, but they varied in diameter from plant to plant. Only tissues from the same plant are thus directly comparable, which was of course true before the cylinders were cut, but there is no need to compare the tissue of one plant with that of another, for the present purpose.

Although I know of no experimental evidence to substantiate the supposition, it appears reasonable to suppose that a given piece of tissue may show a different water-holding capacity at 15.6°C. (60°F.) from that shown at 32.2°C. (90°F.). Consequently, to obtain the water-holding capacity under conditions as near as possible like those which existed in the plant at the time of sampling, the absorbing tissue was allowed to take up water at the temperature to which it had just been subjected in the plant.

Weighings were taken at much shorter intervals than in the former experiments and it was found that the major amount of absorption took place within the first 10 minutes; unless it is otherwise stated, the amounts given in table 12, which presents these data, are for that period. Thus, in this

experiment, the water-holding capacity for equal times is measured and by this means an element of rate is introduced. If there is a difference in rate it should be of importance in determining the final effect of the water-holding capacity on transpiration and absorption.

*Results:* As in experiment 9, the tissue from plants kept below 26.7°C. (80°F.) at night shows a greater original water content and a greater water-absorbing as well as water-holding capacity for the day than for the night. The tissue from plants held at 35°C. (95°F.) at night shows either the same or a greater water-absorbing and water holding-capacity for the night than

TABLE 12

*Comparative differences, between 10 a.m. and 12 night, in the water-holding capacity of tissue from 2 sets of plants, one set kept under typical conditions during both the day and night, the other exposed to typical conditions during the day and at night held at 35.0°C. (95°F.). Values are expressed as grams per gram of dry weight. (Experiment 10.)*

	IN OPEN ALL NIGHT, TEMP. 23.9°-18.3°C. (75°-65°F.)			IN CAGE ALL NIGHT TEMP. 35°C. (95°F.)		
	Plant No.	10 a.m.	12 night	Plant No.	10 a.m.	12 night
Original water-content.....	1	13.6	12.1	2	13.9	17.7
Water absorbed in 10 minutes.....		1.8	0.5		0.9	1.7
Maximum water-content after 10 minutes.....		15.4	12.6		14.8	19.4
Original water-content.....	14	11.3	10.8	3	18.6	19.8
Water absorbed in 30 minutes.....		2.2	0.05		2.8	1.4
Maximum water-content after 1st half-hour...		13.5	10.8		21.4	21.2
Original water-content.....	14	11.3	10.8	15	15.8	17.2
Water absorbed in 10 minutes.....		2.2	0.1		2.3	1.5
Maximum water-content after 10 minutes.....		13.5	10.9		18.1	18.7
Original water-content.....	20	11.7	10.3	13	13.3	13.3
Water absorbed in 10 minutes.....		0.3	0.1		0.4	0.2
Maximum water-content after 10 minutes.....		12.0	10.4		13.7	13.5

for the day. One of the plants used showed a much higher original water-content than the others, and both the day and the night samples lost weight during the first 10 minutes in water. However, the same plant showed a higher water-content after a night at 35°C. (95°F.) than it did for the previous day.

When the results of this experiment are compared with the variations in  $T/E$  and  $A/T$ , for the same conditions, it is seen that the water-holding capacity varies as in experiment 9, directly with  $A/T$  and indirectly with  $T/E$ . It is very evident that an interruption of the typical night temperatures interrupts the normal sequence in the changes of the water-holding capacity of the tissues, just as it interrupts the variations in the values of  $T/E$  and  $A/T$ .

It will be remembered that experiment 3 showed that plants in a desiccated condition do not show the typical day to night variation in  $A$ ,  $T$  and  $T/E$ , and that the direction of these variations may even be reversed in such plants. The next experiment deals with variations in the water-holding capacity of desiccated tissue.

### Experiment 11

*Object:* The determination of the variations in water-holding capacity between day and night, in desiccated tissue.

*Material:* Tissue from 5 potted plants. No. 30 had been without water for somewhat over six months and the others had not been watered for three weeks.

*Method:* The details of the procedure were the same as in experiment 9. In the case of Nos. 1 and 4, small cylinders were cut longitudinally from the joint. Each sample included material from within and from without the vascular ring, both vascular tissue and epidermis being excluded. The

TABLE 13

*Diurnal changes in water-holding capacity of desiccated tissue; values are expressed as grams per gram of dry weight. (Experiment 11.)*

	FIRST HOUR		TOTAL		PLANT NO.
	5:30 p.m.	5:30 a.m.	5 p.m.	5 a.m.	
Original water-content.....	10.3	11.3	10.3	11.3	29
Water absorbed.....	3.2	2.5	4.9	5.0	
Maximum water-content.....	13.5	13.8	15.2	16.3	
Original water-content.....	10.3	9.6	10.3	9.6	1. joints 1 yr. old.
Water absorbed.....	1.8	2.1	2.1	2.7	
Maximum water-content.....	12.1	11.7	12.4	12.3	
Original water-content.....	7.1	6.6	7.1	6.6	1. joints 4 yrs. old.
Water absorbed.....	0.8	1.2	1.7	1.7	
Maximum water-content.....	7.9	7.8	8.8	8.3	
Original water-content.....	8.8	9.5	8.8	9.5	4
Water absorbed.....	2.2	2.0(?)	4.4	4.0	
Maximum water-content.....	11.0	11.5(?)	13.2	13.5	
Original water-content.....	10.6	10.6	10.6	10.6	28
Water absorbed.....	0.8	1.1	3.2	3.9	
Maximum water-content.....	11.4	11.4	13.8	14.3	
Original water-content.....	2.0	2.5	2.0	2.5	30
Water absorbed.....	2.1	1.6	2.8	2.3	
Maximum water-content.....	4.1	4.1	4.8	4.8	

material from No. 28 was cut in cross section and included all material with the exception of the epidermis. Large longitudinal cylinders were cut from No. 30, and each one included all the tissues excepting the epidermis. All three of the previously used methods were thus combined in this experiment.

*Results:* Data appear in table 13. No changes from day to night occur in either the water-holding or the water-absorbing capacity. A comparison of the original water contents in this experiment, with the values found in experiments 9 and 10, shows that the original water content is lower in the desiccated tissue, and that the amount of water absorbed by a gram of entire tissue from a plant desiccated for 6 months is about 5 times as much as that absorbed by the same weight of tissue from a plant not so desiccated. (The absorption per gram of *dry weight* is about the same in the two cases). Long<sup>11</sup> found the same phenomena to be true for platyopuntias; his experiments show an increase of 29 per cent. in the amount of water-intake after desiccation in the open.

Experiment 11 adds another bit of evidence in favor of the idea that  $T/E$ ,  $A/T$ , and the water-holding capacity of the tissues have a constant inter-relation, no matter what the influence of the environment may be on them. On page 103 it was suggested that changes in the water-holding capacity of the tissue ought theoretically to affect the values  $T/E$  and  $A/T$ . It has now been found, not only that such changes in the water-holding capacity do actually occur, but also that they have a definite relation to the variations in  $T/E$  and  $A/T$ . The mechanism of the relation may be pictured as follows.

#### EFFECT OF VARIATIONS IN WATER-HOLDING CAPACITY OF TISSUES ON $T/E$ AND $A/T$

As water is lost by transpiration from the exposed cell walls surrounding the intercellular spaces, it is replaced by water, first from the protoplast within and then from neighboring cells, provided the imbibitional and osmotic forces in the cells depleted by transpiration are greater than these forces in the neighboring cells. For the sake of brevity, let the region of surfaces exposed directly to transpirational loss be termed  $a$ , let the region immediately back of, and bordering on  $a$ , be termed  $b$ , and let the region between the soil and  $b$  be termed  $c$ . If  $a$  and  $b$  were alone present, the rate of passage of water from  $b$  to  $a$  would be determined by the resultant of the forces tending to hold water in  $b$  and those tending to make water pass from  $b$  to  $a$ ; but since  $b$  can draw upon  $c$  for a further supply of water, this rate of entrance to  $a$  must be influenced also by the forces which tend to make water pass from  $c$  to  $b$ . Suppose that, at a given instant, equilibrium is established with water entering  $a$  at a slightly slower rate than it is being taken out by tran-

<sup>11</sup> MacDougal, D. T., Long, E. R., and Brown, J. G., End results of desiccation and respiration in succulent plants. *Physiol. Rev.* 1: 280-325. 1915.

spiration, and that, at the next instant, the water-holding capacity of *b* is decreased, then the following instant would show water entering *a* faster than before and entering *b* from *c* at a slower rate. The net result would, in this case, be a lower water-content for *b* and a higher water-content for *a*, provided, of course, that no other conditions changed. If, on the other hand, an increase in the water-holding capacity of *b* had occurred, instead of a decrease, the following instant would have shown a decrease in the rate of entrance to *a* and an increase in the rate of entrance to *b* from *c*. The net result this time would have been an increase in the water-content of *b* and a decrease in that of *a*.

If Livingston and Hawkins [1915] are correct in stating that "with a given [cell] wall, the more nearly it approaches saturation with water, the more nearly must its transpiring power approach a maximum," we see how a change in water-holding capacity of internal tissue can operate to cause changes in transpiration. Also, since a change in water-holding capacity of *b* can cause a change in the rate of entrance of water from *c* to *b*, it will necessarily cause an increased rate of entrance from the soil to *c*,—unless there appear other forces strong enough to reverse the resultant. *b* and *c* might act either independently or as a unit, but the final effect on transpiration and on absorption would be the same.

To prove that changes in water-holding capacity of internal tissue directly influence the rate of water loss from the exposed surfaces, an extensive separate research would have to be performed. This I have not done, but I offer the next experiment as evidence that the theory advanced in the last paragraph may be tested by experiment.

### *Experiment 12*

*Object:* A comparison of the evaporating power of naked internal tissue with the water-absorbing capacity of the same tissue.

*Material:* Internal tissue of joints one year old, from an adult plant growing in the open.

*Method:* By means of a cork-borer cylinders of uniform size were cut across the joints, the epidermis being afterward removed. As the cylinders were cut, alternate ones were taken for the absorption and for the evaporation tests. Each set of tests represents material from a single joint. The material for studying absorption was placed in distilled water and treated in all respects as in experiments 11 and 12. The cylinders for the study of evaporation were placed in parallel rows at equal distances apart in shallow weighing bottles. Into other weighing dishes, of exactly the same size and shape, water was poured to a height equal to the depth of the cylinders occupied in their dishes. These dishes were all placed side by side in a glass chamber where the temperature and humidity were held constant. The water loss

from the tissues for the first hour was expressed as loss per gram of original wet weight, and this was then divided by the loss from the water surface for the same period; this quotient is here called the evaporating power of the tissues.

*Results:* Data are presented in graphical form in figure 9. On the lower graph (A) are points representing the absorbing capacity in the order of

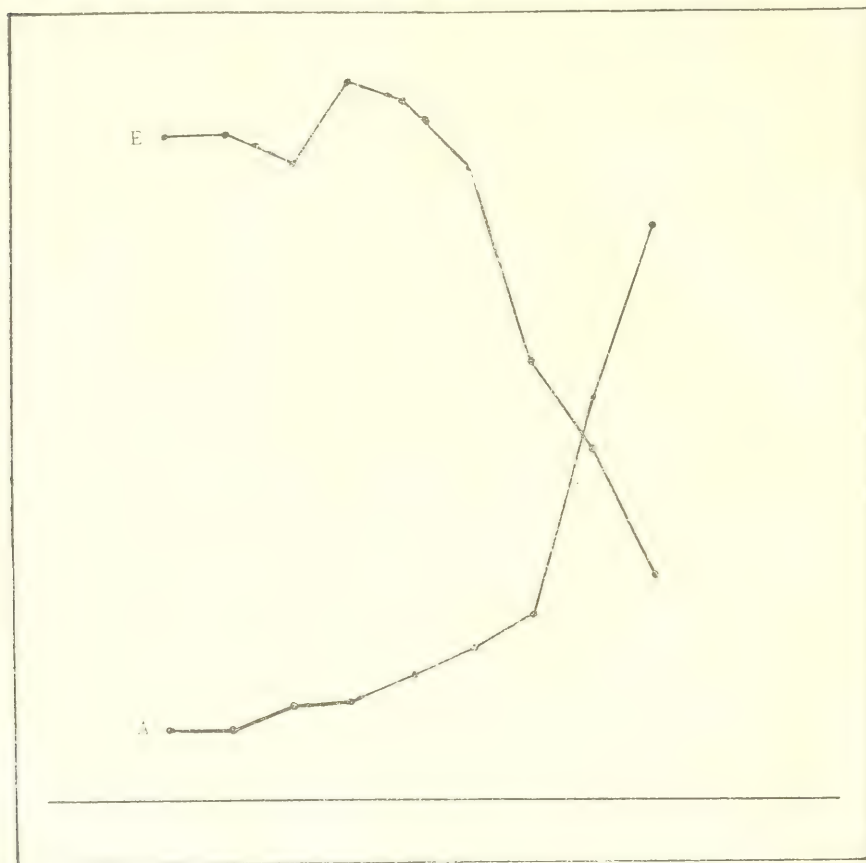


FIG. 9. Comparison of evaporating power of naked tissue with water-absorbing capacity of the same tissue. (See experiment 12.)

increasing magnitude; on the upper graph (E) are shown the corresponding values for the evaporating power. The samples were taken at various times during a 24-hour period, but this order does not appear in the graph. The absolute absorption rate is considered instead of the water-holding capacity, since the object here is to discover any relation that may exist between the amount taken in and the amount lost by evaporation, regardless of the cause

of the changes in absorption. Since only material from the very same joint is compared, variations in water-content between the several joints would not influence these comparisons.

The first five points in the graphs give an idea of the unexplained variations which may be expected, and the remaining four points show the changes in evaporating power, with increasing absorbing capacity of the tissue. It is very evident that the last four changes are greater than the variability, and that the evaporating power decreases as the absorbing capacity increases. This is according to prediction; but, as was emphasized above, this experiment is by no means extensive enough to establish this relation.

Evaporation from small pieces of living tissue is not the same phenomenon as evaporation from a weak gel solution and hence the behavior found in this experiment does not necessarily offer a contradiction to conclusions of previous workers<sup>12</sup> who have shown that the water lost from a weak gel solution (of gelatine) is approximately the same as that lost from a similar water surface. I do not suggest that it is an increase in sap concentration that here causes the observed decrease in evaporation rate.

Theoretically at least, we may suppose that the changes in water-holding capacity influence directly the changes in  $T/E$ , but there is another phenomenon which must not be overlooked, namely possible changes in the stomatal apertures. The next experiment is concerned with the measurement of stomatal movements in the plant here studied.

### *Experiment 13*

*Object:* The measurement of stomatal openings under conditions which produce (1) typical and (2) atypical behavior with respect to  $T/E$ .

*Material:* An adult plant growing in the open (A) and four potted plants transplanted from the open about one year before this experiment (Nos. 2, 38, 39 and 40).

*Method:* Lloyd's [1908] method of plunging peeled epidermis into absolute alcohol was used. It was not possible to strip off the epidermal layer alone, since the natural line of cleavage occurs at a depth of from three to four cells in this plant. The cells immediately below the true epidermis are heavily-walled and non-chlorophyllous, and between them are narrow canals leading from the larger intercellular spaces below to the guard cells, which are situated at the base of the true epidermal layer. It is probable that no serious error was introduced by the inclusion of these cells with the true epidermis since they contained a low percentage of water and since Livingston and Estabrook<sup>13</sup> have shown that the presence of as much as 10 per cent. of water in

<sup>12</sup> Long, E. R., in: MacDougal, Long and Brown [1915], Page 209.

<sup>13</sup> Livingston, B. E. and Estabrook, A. H., Observations on the degree of stomatal movement in desert plants. Bull. Torr. Bot. Club 39: 15-22. 1912.

the alcohol used does not bring about closure of stomatal apertures. The values for different times of the day are surely comparable. The pieces were stained with Congo red in absolute alcohol, and then mounted in balsam, before they were examined with the microscope.

The potted plant No. 38 and a large limb from the adult bush C were placed for the night in a constant temperature cage at 33.9°C. (93°F.). The potted plant No. 39 and the large bush A were left at all times under natural conditions in the open. In the case of plant A samples were taken from 3 joints and were kept separate, for comparison. For the other plants samples were taken from several joints but were all placed on the same slide and the sizes of their apertures were averaged.

TABLE 14

*Average stomatal apertures (square-root of the product of length times width of opening), in micra, of 5 plants under two different conditions of nocturnal temperature. (Experiment 13.)*

TIME	NIGHT TEMPERATURE, 7.2°-17°C. (45°-35°F.)				NIGHT TEMPERATURE, 32.2°-35°C. (90°-95°F.)	
	Plant E		No. 2	No. 38	No. 39	No. 40
	Branch 1	Branch 2				
12 noon	closed	closed	closed	closed	closed	closed
3 p.m.	4.87, many closed	5.96, many closed	closed and 4.86	closed and 5.17	closed	closed and 2.98
6 p.m.	6.87	5.96	closed and 5.96	6.90	closed <sup>a</sup>	closed <sup>a</sup>
9 p.m.	3.45	5.96	6.67	9.32	closed and 5.08	closed and 5.14
3 a.m.	7.54	7.70	6.67	10.09	closed	closed
7 a.m.		7.78	4.62	9.03	closed	closed
9 a.m.	4.84	9.22	7.70	9.03	closed and 4.87	closed, and 4.19
12 noon	closed	closed	closed	closed	closed	closed

<sup>a</sup> Nos. 39 and 40 were placed in constant temperature cage at 5 p.m.

*Results:* The data appear in table 14. Each number given is the average of 20 apertures consisting of 18 pores of the estimated average size and one of each of the extremes. Under typical conditions all of the plants had closed stomata during the day and open ones at night. When the plants were kept at about 32.2°C. (90°F.), at night, the stomata remained closed. Under typical conditions the stomata begin to close soon after sunrise and

they appear to be all completely closed by noon. They begin to open at from 5 to 6 o'clock in the evening and continue to open throughout the night, the maximum size occurring between 3 and 6 a.m.

In these stomatal movements we see an unquestionable influence in the control of  $T/E$ , and, at least until further evidence appears, we may, if we like, regard these changes in aperture as entirely responsible for the variations in the value of this ratio. However, the causes of these unusual variations in aperture remain to be determined. So far as I know, no other plants have yet been recorded as showing stomata consistently closed during the day and open at night. From figure 10 comparison may be made of the stomatal movements (ST) and the changes in water-holding capacity of internal tissue (Max. W. C.). The aperture is largest when the water-holding capacity is least and vice versa. Now, since the size of the opening varies directly with the turgidity of the guard cells, it appears that the water-holding capacity of the internal tissue should vary inversely with the turgidity of the guard cells.

Using the same designations as in a previous paragraph we may place the guard cells in region *a* and the internal tissue in region *b*. Starting with a condition of equilibrium in which water is being conducted to *a* at the same rate that *a* is losing water by evaporation, suppose that at the next instant, the water-holding capacity of *b* increases, while all other conditions for *a* remain the same. There should follow a decreased rate of entrance of water to *a* from *b*, causing a net decrease in the water-content of *a*. If this condition continues for several successive intervals of time the water-content of *a* should become so low that the guard cells would lose their turgidity and the stomata would close. They must then remain closed until the rate of entrance of water exceeds the rate of loss by evaporation. Supposing still that all other conditions remain constant, then a decrease in the water-holding capacity of the region *b* should allow a more rapid entrance of water to *a* and a net gain of water would result for *a*, and if these conditions continue long enough the guard cells should open. Under some circumstances a large enough increase in the water-holding capacity of *b* might even cause a transfer of water from *a* to *b* and a subsequent decrease in the water-holding capacity of *b* might cause a return of the excess to *a*. The actual relation in the plant is of course by no means so simple as the above analysis supposes, because neither the evaporative forces of the air nor the osmotic forces of the guard cells remain constant.

If, as has been supposed, it is an increase in the osmotic pressure of the guard cells which, for the non-succulent plant, causes the stomata to open in the morning, by bringing about a greater rate of water entrance to the guard cells, then the increased osmotic pressure may be overbalanced in that plant by an increase in the water-holding capacity of the tissue within. On the other hand, the decrease in osmotic strength which has been generally postu-

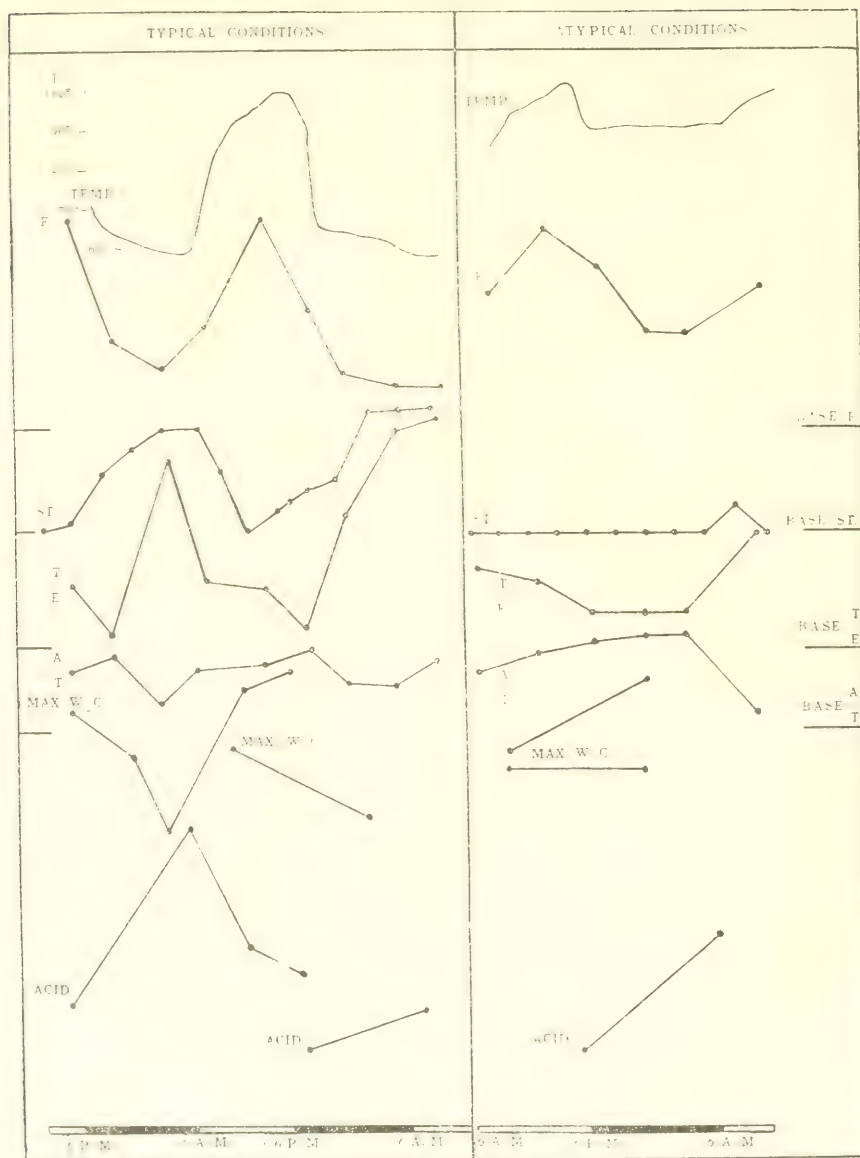


FIG. 10. Selected graphs showing marches of various phenomena under typical and atypical environmental conditions.  $T/E$  and  $A/T$  were determined simultaneously, as were also the maximum water-content (i.e., water-holding capacity) and acidity. All graphs in each group indicate behavior under identical conditions.

lated to explain stomatal closure at night may be counteracted in the cactus by the nocturnal decrease in the water-holding capacity of the internal tissues. This theory seems to offer an explanation for the unusual behavior of the stomata of this plant; but it will also be observed that the changes observed in the water-holding capacity of the tissues are probably sufficient to offer a theory for the lower  $T/E$  in the day time. It is possible that a study of the water-holding capacity of the tissues of non-succulents might throw some light on the behavior of their stomata also.

It has appeared thus far that a decrease in the water-holding capacity of the tissue may cause an increase in the value of  $T/E$ , both by reason of its direct effect on the water loss from the cells surrounding the intercellular spaces and by reason of its action on the guard cells. A comparison of  $T/E$  per unit of area with the calculated amount of diffusion possible through the openings at various times might show that the stomatal movements alone were sufficient to account for the variations in  $T/E$ . So far, there are two serious difficulties in the way of such a comparison, for it is impossible to obtain an accurate measurement of the area of the plants by any of the known methods, and  $T$  and  $E$  cannot be measured in the same units of area. Other difficulties nearly as serious arise from the facts, that transpiration and stomatal measurements cannot be made for the same plant at the same time, and that the change in size of the stomatal aperture due to the alcohol treatment is not yet known.

#### PRELIMINARY INVESTIGATION OF SOME OF THE CAUSES OF THE VARIATIONS IN WATER-HOLDING CAPACITY OF THE TISSUES

If we accept the changes in the water-holding capacity of the tissues as a major factor in the control of the variations in transpiring and absorbing power, then, evidently, the next step in the analysis must be the discovery of the cause of the variations in the water-holding capacity. In the light of recent work on the influence of acids on the swelling of colloids, it seemed possible that the large changes in the acidity of this cactus, found by Richards<sup>14</sup> and Spoehr<sup>15</sup> might be responsible for the changes in the water-holding capacity here dealt with. If this is true, then the influence of light and temperature conditions on acidity ought to parallel their influence on the water-holding capacity of the tissues. From experiment 10 it appears that the water-holding capacity does not decrease on a night when the temperature is held at 32.2°C. (90°F.) but does do so when the temperature is below 26.7°C. (80°F.). Now, if an increase in acidity is directly responsible for a

<sup>14</sup> Richards, H. M., Acidity and gas exchange in cacti. Carnegie Inst. Wash. Pub. 209. Washington, 1915.

<sup>15</sup> Spoehr, H. A., Photochemische Vorgänge bei der diurnalen Entsäuerung der Succulenten. Biochem. Zeitschr. 57: 95-111. 1913.

decrease in the water-holding capacity then the acidity of material which has had the same immediate previous history ought to show at least a smaller amount of acid increase at 32.2°C. (90°F.) than at 15°–21°C. (60°–70°F.). Richards says, in the conclusions of his publication, "after a cool night the acidity [of *Opuntia versicolor*] is measurably higher than after a warm one," but in the text of his paper (page 30, 31) he says regarding the increment of acid in the dark, in plants where the amount is already low: ". . . . of the three temperature ranges employed, the accumulation of acid in both young and old tissue is greatest at 30°C. and least at 40°C. . . . . mature joints with an average initial acidity of 0.24 per cubic centimeter juice show accumulation of acid after 18½ hours to the following amounts: at 20°C., 1.15; at 30°C., 1.54; at 40°C., 0.62. Similarly the younger parts, which had an average initial acidity of 0.24 per cubic centimeter juice showed during the same period the following gain: at 20°C., 1.44; at 30°C., 1.74; at 40°C., 0.75." (Amounts are cc. of N/10 alkali.) Consequently it seemed best to test the acidity changes for the material in question under the exact conditions dealt with, and the three following experiments were undertaken with this end in view.

#### *Experiment 14*

*Object:* A determination of the changes in acidity which occur in limbs exposed to a constant temperature of 32.2°C. (90°F.) for the night hours; and a comparison of these results with the changes in acidity occurring in other limbs from the same plant, exposed to the natural, low night temperatures.

*Material:* Year-old joints from a large bush growing in the open. The bush was probably between 25 and 35 years old.

*Method:* At 6 p.m., at the close of a cloudless, hot day, when acidity was presumably low, samples were taken directly from the plant and used immediately for titration. At 5:30 p.m. four limbs were removed from the plant, so that the severed limbs each included from 8 to 10 joints below the joints which were to be used. Two of the limbs were left on the open porch until 5:30 a.m., when samples were taken. The other two were placed, for the night in a darkened cage where the temperature was held at 32.2°C. (90°F.). The limbs in the cage were kept in darkness until the samples were taken, at 8 a.m. As a check on the limbs left on the porch samples were taken at 4 a.m. from limbs which had not been severed from the plant.

The method for the extraction of the juice, which was in all essential respects the same as that used by Spöchr, was briefly as follows. The entire joints were first put through a meat-grinder and all of the material taken at a given time was placed in a large cheesecloth sack. This sack was then pressed with a small wine-press and the juice was collected in a graduated cylinder. The press was opened three times and the contents of the sack were stirred,

and pressing was repeated after each process of stirring. At each pressing the screw was turned as far as possible, and thus a constant pressure treatment of the material was approximated. The pulp was saved and its dry weight was determined later. About 300 cc. of juice was always obtained. Each lot of juice was weighed in the graduated cylinder in order to obtain data for the determination of density. A known amount of water was added, until the volume was increased to four times its original amount. This diluted juice was heated over water to a temperature of 84°C., when the slime was all precipitated. Then 25 cc. was drawn off with a pipette, while still hot, and diluted to 10 times its volume, with hot, freshly boiled, distilled water. It was immediately titrated against N 50 NaOH. Deter-

TABLE 15

*Acid content of juice from one year old joints which have been exposed to darkness and natural night temperatures, as compared to similar content of juice from joints which have been exposed to darkness and a controlled temperature of 32.2°C. (90°F.) Experiment begun at the close of a cloudless, hot day. (Experiment 14.)*

DATE	TIME	AMOUNT OF N/50 NaOH REQUIRED TO NEUTRALIZE 1 CC. OF UN- DILUTED JUICE	DENSITY OF JUICE (WATER = 1)	NOTES
		cc.		
May 11...	6:00 p.m.	1.37	1.037	Joints cut directly from plant.
May 12...	4:00 a.m.	2.01	1.042	Joints cut directly from plant. Night temp. 23.9°-16.7°C. (75°-62°F.)
May 12...	5:30 a.m.	2.86	1.034	Joints cut from limbs which were severed at 5:30 p.m. Night temp. 23.9°-16.7°C. (75°-62°F.)
May 12...	8:00 a.m.	4.73	10.41	Joints cut from limbs which were severed at 5:30 p.m. Controlled temperature, 32.2°C. (90°F.)

minations were made with a test plate, using phenolphthalein as indicator. Ten samples were titrated from each lot of juice. A little practice made it easy to obtain an agreement between the samples within 2 per cent. As will be seen from the tables which follow, this error is insignificant in the light of the large differences in acidity which were obtained.

*Results:* Table 15 presents the equivalents in cc., of N 50 NaOH for 1 cc. of undiluted juice. It will be seen that not only do the limbs left at the natural, low night temperatures increase in acidity but so also do those held at the higher temperature during the night. The material held at the higher temperature showed a greater increase in acidity than did the material left in the open.

*Experiment 15*

*Object:* The same as for experiment 14.

*Material:* Year-old joints from an adult bush growing in the open. The bush was probably between 20 and 30 years old.

*Method:* The same as that used in the last experiment, with the following exception. The severed limbs were kept for a longer time under the controlled conditions, one being kept at 16.7°-15.6°C. (65°-60°F.) in darkness, from 5 p.m. until 9 a.m. and the other, also in darkness, but at 32.2°C. (90°F.), from 5 p.m. until noon.

*Results:* Data appear in table 16. The longer period shows a still greater increase of acidity, under the high temperature conditions, than in the preceding case.

TABLE 15

*Acid content of juice from year-old joints which have been exposed to darkness and natural night temperatures, as compared with acidity of juice from similar joints which have been exposed to darkness and a controlled temperature of 32.2°C. (90°F.) Experiment begun at the close of a cloudless, hot day. (Experiment 15.)*

DATE	TIME	AMOUNT OF N/50 NaOH REQUIRED TO NEUTRALIZE 1 CC. OF UN- DILUTED JUICE	NOTES
		cc.	
May 10.....	6 p.m.	1.57	Joints cut directly from plant.
May 11.....	5 a.m.	2.81	Joints cut directly from plant. Night temperature 21.1°-14.4°C. (70°-58°F.)
May 11.....	9 a.m.	3.72	Joints cut from limbs which were severed at 6 p.m. and kept in dark at 21.1°-15.6°C. (70°-60°F.) until used.
May 11.....	12 noon	5.94	Joints cut from limbs which were severed at 6 p.m. and kept in dark at 32.2°C. (90°F.) until used.

*Experiment 16*

*Object:* The same as for experiments 14 and 15.

*Material:* Year-old joints from an adult bush growing in the open. The bush was probably between 15 and 20 years old.

*Method:* All samples were taken directly from limbs in situ. This was accomplished by placing one half the bush under a glass cage containing the thermal elements and automatic control, electric current being obtained from the laboratory. As before, a record of the humidity within and without the cage was taken and the relative value in the two places was found to vary less than 10 per cent. The other half of the bush remained exposed to the

natural conditions of the night. Thus all errors that might be due to the use of severed limbs were avoided.

*Results:* The data appear in table 17. The results are essentially the same as when severed limbs were used, namely, more acid accumulated at 32.2°C. (90°F.) than at 21.1°–15.6°C. (70°–60°F.).

In the last three experiments it was necessary to use large adult plants, in order to obtain sufficient material to use the method of juice-extraction which had been employed by Spoehr and by Richards. Several sets of tests were made for the water-holding capacity of the tissue of large adult plants and the results were found to agree with the tests made on the potted plants used in the transpiration experiments. It is therefore entirely permissible to

TABLE 17

*Acid content of juice from year-old joints which have been exposed to darkness and normal night temperatures as compared with the acidity of similar joints which have been exposed to darkness and a controlled temperature of 32.2°C. (90°F.). All joints were left on the plant until the samples were taken. Experiment begun at the close of a cloudless hot day. (Experiment 16.)*

DATE	TIME	AMOUNT OF N/50 NaOH REQUIRED TO NEUTRALIZE 1 CC. OF UN- DILUTED JUICE	DENSITY OF JUICE (WATER = 1)	NOTES
		cc.		
May 15..	5 p.m.	1.94	1.048	
May 16..	4 a.m.	2.95	1.050	From limbs which had been under natural temperatures of 23.9°–16.7°C. (75°–62°F.)
May 16..	5 a.m.	4.90	1.050	From limbs which had been exposed to a constant temp. of 32.2°C. (90°F.)

compare the results of the experiments on water-holding capacity with these data on acidity. Nevertheless, it may not be justifiable to compare the acidity data with the transpiration results obtained with potted plants, and so it seemed desirable to obtain tests for water-holding capacity and for acidity simultaneously, on the same plants as had been previously used in the transpiration experiments. The method used in the next experiment, for the extraction of juice, is one used by Long (MacDougal, Long and Brown [1915]). I have applied several rather rigorous tests to this method and have found that, although it is by no means as accurate as one where large quantities of juice are employed, nevertheless, the error is of little importance in the determination of changes in acidity taking place in a period of 6 hours.

*Experiment 17*

*Object:* A determination of the acidity of joints of the same plants as were used in experiment 10, under both typical and atypical conditions.

*Material:* Year-old joints taken from plants H and Nos. 1, 2, 3, 4, 13, 14, 15, and 20.

TABLE 18  
*Variations in acidity of juice from plants exposed to typical conditions.*  
(*Experiment 17*)

TIME	PLANT	JOINT	AMOUNT OF N. 50 NaOH REQUIRED TO NEUTRALIZE ACID FROM 1 GRAM, DRY WEIGHT		AMOUNT OF N. 50 NaOH REQUIRED TO NEUTRALIZE ACID FROM 1 GRAM, WET WEIGHT	
			Center	Periphery	Center	Periphery
			cc.	cc.	cc.	cc.
12 noon	H		41.8	50.5	3.84	5.71
5 p.m.	H	1	18.8	32.8	1.52	3.71
5 p.m.	H	2	.....	31.7	1.69	3.68
5 p.m.	H	3	.....	38.2	2.54	3.72
6 a.m.	H	1	75.5	87.0	5.32	10.23
6 a.m.	H	2	76.8	80.4	5.06	7.95
12 noon				71.6	.....	.....
5 p.m.	2			32.8	.....	4.14
6 a.m.	2			90.7	.....	12.30
12 noon	2				.....	5.70
5 p.m.	2				.....	3.90
5 p.m.	4	1		31.7	.....	3.90
5 p.m.	4	2		38.3	.....	5.00
6 a.m.	4	1		80.4	.....	9.40
6 a.m.	4	2		87.4	.....	10.61
12 noon	4					6.05
5 p.m.	4					4.83

*Method:* Plants were left in the green-house during the day, and at night joints from one-half of the plant were led into a constant temperature cage, through a suitable opening in the side. After the limbs were in position the opening was closed by a sectioned board and a wad of cotton, so that very little circulation of air took place through the opening.

Cylinders were cut from internal tissue exactly as in experiment 9, longitudinal cylinders being cut from both center and periphery. These were immediately placed in 98 per cent. alcohol, where they remained in a cool dark place until they were used, which was 2 hours after they were placed in the alcohol. The method used for the determination of the acid (already employed by Long, as has been mentioned) is based on a hot alcoholic extraction of the acids, all of which are soluble in alcohol. A Soxhlet extraction shell was fastened to the lower part of a reflux condenser; surrounding the shell was a large-mouth flask, fitted to the condenser by an air-tight stopper. The cylinders were taken from the alcohol and placed in the shell and the

TABLE 19

*Nocturnal variations in acidity of juice from plants exposed to typical and atypical temperature conditions. (Experiment 17)*

TIME	PLANT	LOT	AMOUNT OF N 50 NaOH REQUIRED TO NEUTRALIZE ACID FROM 1 GRAM, DRY WEIGHT	NOTES
			cc.	
5 p.m.	3	1	30.5	At close of hot, cloudless day.
5 p.m.	3	2	33.8	
4 a.m.	3	1	81.9	Exposed to darkness, 16.7°—21.1°C. (65°–70°F.)
4 a.m.	3	2	78.4	
5 a.m.	3	1'	95.1	Exposed to darkness, 32.2°C. (90°F.)
5 a.m.	3	2'	90.3	
5 p.m.	20	1	32.3	At close of hot, cloudless day.
5 p.m.	20	2	29.8	
4 a.m.	20	1	79.1	Exposed to darkness, 16.7°–21.1°C. (65–70°F.)
4 a.m.	20	2	76.3	
5 a.m.	20	1'	88.3	Exposed to darkness, 32.2°C. (90°F.)
5 a.m.	20	2'	92.3	

alcohol was poured into the flask beneath. The bottom of the shell was several centimeters from the surface of the alcohol in the flask. The alcohol in the flask was then kept boiling for an hour, over a water bath, cold water being passed through the outer jacket of the condenser during the boiling. At the close of the hour the condenser was washed with freshly-boiled alcohol, the rinsings passing into the flask below. The alcohol, while still hot, was titrated against N 50 NaOH, phenolphthalein being used as indicator.

*Results:* The data appear in tables 18 and 19. Table 18, which gives the changes in acidity when the plants were subjected to typical conditions, shows the same general results as were obtained by the former method of

extraction of juice. In both tables 18 and 19 the acidities have been reduced to acid equivalents per gram of dry weight and, in table 18, per gram of original wet weight as well; the conclusions are the same, whichever basis is used for comparison. Table 19 furnishes a comparison of the nocturnal changes in acidity at 32.2°C. (90°F.), with those at 16.7°–21.1°C. (65°–70°F.), and shows,—just as the former method did,—that more acid is accumulated at the higher temperature than at the lower.

A comparison of the results of the last four experiments with those of experiment 10 reveals the fact that under "typical" conditions the water-holding capacity of the tissue is highest when acidity is lowest, and vice versa. It will be noted that plant H, in table 18, shows always a higher acidity for the periphery than for the center; the same is true for the acidity test on six other plants, the data for which do not appear in the tables. Now, from table 11 (experiment 10) it is seen that the periphery of a given joint has a lower water-holding capacity than the central tissue. Here is further evidence, therefore, that low absorbing and water-holding capacity and high acidity occur simultaneously, and that low acidity and high absorbing and water-holding capacity are associated. This relation is the opposite of the one found by Fischer<sup>16</sup> and others, as regards the effect of acid on the swelling of certain colloids. Long,<sup>17</sup> however, found that cactus joints (*Opuntia blakeana*) swell more in distilled water and in M/100 NaOH than in M/50 malic acid or M/100 HCl.

The effect of acids upon the water-holding capacity of plant tissues is by no means well understood as yet, but even a settlement of the question thus raised would not give a final answer to the problem considered here; that more acid accumulates at night, at 32.2°C. (90°F.), than at 21.1°–15.6°C. (70°–60°F.), shows that it is not possible to regard changes in hydrogen-ion concentration alone as the cause of the variations in water-holding capacity.

While the results of these last experiments complicate the question of the relation between water-holding capacity and acidity, they do not prove that acid has no influence upon the absorption and water-holding capacity of the tissue. On the one hand, it is quite possible that different concentrations of acid may have different effects upon the water-holding capacity, and thus that the greater amount of acid which accumulates in the tissues at the higher temperature, at night, may increase the concentration enough to reverse the effect of the acid. On the other hand, it seems more probable that various substances within the tissue may exert opposing influences on the water-holding capacity, the final effect being determined by the resultant of all, so that, at the higher temperature, the balance may be swung to the other side by a change in amount of one or more of the substances present.

<sup>16</sup> Fischer, M. H., *Oedema*. New York, 1910.

<sup>17</sup> Long, F. R., Growth and colloid hydration in cacti. *Bot. Gaz.* 59: 491-496, 1915.

It is well known that acids, bases, and neutral salts affect the distribution of water between the disperse and continuous phases of emulsoids (lyophiles). If the tissue of *Opuntia versicolor* may be considered to be composed of cellulose walls surrounding a protoplasmic emulsoid, then it is easy to imagine that the continuous alteration of the chemical constituents of the living tissue is ever causing variations in the distribution of water between cell walls, cell sap, and cell membranes, and between cell walls and soil water as well. Thus these chemical changes may be supposed to be responsible for variations in the water-holding capacity of the whole plant and, through this, for variations in root absorption and in transpiration. In this case the energy needed for the absorption of water by the roots could be traced directly to the imbibitional properties of emulsoids and of cell walls.

The above suggestion is given as one that has arisen out of the investigation here reported. The experiments themselves have shown merely that the same external conditions which influence metabolism influence also the water-holding capacity of the tissues as a whole, and that one of the most characteristic chemical variations in this plant, its diurnal change in acidity, is not independently responsible for the variations in water-holding capacity, although apparently related to these variations. A further, and more complete, investigation of the influence of the normal chemical changes occurring in the plant, on the water-holding capacity of the tissues, would doubtless be very interesting.

It is not evident that the water-holding power of the tissue is controlled by imbibitional properties alone; in fact it seems that variations in osmotic pressure within and without the cell must necessarily cause variations in this capacity. The range of temperature to which the plants are normally subjected ought surely to bring about variations in osmotic pressure, the greater pressure occurring during the day, and producing a greater water-holding and absorbing capacity for the day than for the night. A night with the same high temperature as prevailed during the previous day would, theoretically, if other things remained the same, cause the osmotic pressure (and consequently the water-holding capacity) to remain high. This agrees with the actual behavior of the water-holding capacity (see experiment 10) and is therefore very probably a contributing cause to the variations in water-holding capacity.

I say contributing cause advisedly, because, during the afternoon hours, while the temperature is still near the maximum, the water-holding capacity frequently decreases. Also, of course "all other things" do not remain the same; the very chemical changes which, in a former paragraph, were mentioned as influencing the distribution of water between the gel phase and free water, must also influence the osmotic pressure of the cell sap and consequently the water-holding capacity of the tissue. So, any interpretation of a detailed study of the influence of the chemical changes within the plant

on the water-holding capacity of the tissues must include a consideration of the effects produced on colloidal gels, on cellulose walls, and on the difference in osmotic strength between cell sap and surrounding liquid, in cell walls and intercellular spaces.

### CONCLUSION

As has been mentioned on a previous page, Livingston has postulated two complex factors which govern the water loss of plants, the aridity of the environment as a whole, and the transpiring power of the plant itself. I have found that there occurs within the internal tissue of this cactus a regular diurnal march of change in the water-holding capacity of the tissues and have offered the theory that these changes are both directly and indirectly responsible, at least in part, for the changes in the transpiring power of the plant and that they are also directly responsible for the secondary absorbing power of the plant. The transpiring power of the cactus is usually greater at night than during the day because the water-holding capacity of its tissues is greater by day than by night; i.e., the plant's power of resistance to the aerial aridity factor is greater by day than by night. The probable source of the energy for this resistance lies in the imbibitional forces of the hydrophile colloids and cell walls, and hence in surface tension forces; that is, in the mutual attraction of the molecules.

A summary of the main facts which have appeared during the course of this investigation is presented in figure 10, where the results are given of various measurements, for both typical and atypical conditions. The main conclusions are presented in the Abstract.

*Desert Laboratory of the Carnegie Institution of Washington,  
Tucson, Arizona.*

### LITERATURE CITED

Numbers in brackets throughout the preceeding pages refer to the year of publication and to the corresponding numbers that follow authors' names in this list.

- BAKKE, A. L., Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. *Jour. Ecol.* **2**: 145-173. 1914.
- BRIGGS, J. J. AND H. L. SHANTZ, The wilting coefficient for different plants and its direct determination. U. S. Dept. Agric., Bur. Plant Ind., Bull. 230. 1912.
- FISCHER, M. H., Oedema. New York 1910.
- LIVINGSTON, B. E., 1906, The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Wash. Pub. 50. Washington, 1906.
- 1907, Relative transpiration in cacti. *Plant World* **10**: 110-114. 1907.
- LIVINGSTON, B. E. AND A. H. ESTABROOK, Observations on the degree of stomatal movements in certain plants. *Bull. Torr. Bot. Club* **39**: 15-22. 1912.
- LIVINGSTON, B. E., AND W. H. BROWN, Relation of the daily march of transpiration to variations in the water-content of foliage leaves. *Bot. Gaz.* **53**: 309-330. 1912.
- LIVINGSTON, B. E., AND LOU A. HAWKINS, The water-relation between plant and soil. Carnegie Inst. Wash. Pub. 204; Washington, 1915.
- LYDD, F. E., The physiology of stomata. Carnegie Inst. Wash. Pub. 82. Washington, 1908.

- LONG, E. R., Growth and colloid hydration in cacti. *Bot. Gaz.* **59**: 491-496. 1915.
- MACDOUGAL, D. T., The water balance of succulent plants. *Carnegie Inst. Wash. Pub.* 141. Washington, 1916.
- MACDOUGAL, D. T., E. R. LONG, AND J. G. BROWN, End results of desiccation and respiration in succulent plants. *Physiol. Res.* **1**: 289-325. 1915.
- RICHARDS, H. M., Acidity and gas exchange in cacti. *Carnegie Inst. Wash. Pub.* 209. Washington, 1915.
- SHREVE, E. B. [1914], The daily march of transpiration in a desert perennial. *Carnegie Inst. Wash. Pub.* 193. Washington, 1914.
- [1915], An investigation of the causes of autonomic movements in succulent plants. *Plant World*, **18**: 297-312, 313-343. 1915.
- SHREVE, F., A montane rain-forest. *Carnegie Inst. Wash. Pub.* 199. Washington, 1914.
- SPOEHR, H. A., Photochemische Vorgänge bei der diurnalen Entsäuerung der Succulenten. *Biochem. Zeitschr.* **57**: 95-111. 1913.



# A PRELIMINARY STUDY OF CLIMATIC CONDITIONS IN MARYLAND, AS RELATED TO PLANT GROWTH<sup>1</sup>

(CARRIED OUT UNDER THE AUSPICES OF THE MARYLAND STATE  
WEATHER SERVICE, IN 1914)

FORMAN T. McLEAN

## ABSTRACT<sup>2</sup>

This study is an attempt to test certain methods for determining some of the quantitative relations between climatic conditions and the growth of plants. Since these relations are very complex, and since the interpretations of experimental results bearing on these relations are exceedingly difficult, the preliminary stages of such interpretations may be advanced by employing the growth rates of a standard plant as a measure of the effectiveness of the surroundings to produce growth. To do this, it is necessary to employ plants that are as nearly alike as possible at the beginning of the various tests. The plant is thus regarded as a sort of integrating and recording instrument, the reading of which is zero at the beginning of each observation period. The plant is allowed to grow during the period, and the effectiveness of the environmental conditions during that time is measured in terms of the amount of growth produced.

This method was employed in these studies, the plant used being soybean. A new observation period began approximately every two weeks and continued for a month, so that the different periods overlapped. Observations on growth were also made at the end of about two weeks; that is, at the middle of the month.

To have the plants of all tests nearly alike at the beginning of the period, they were always started from the seed. Dry seeds change less rapidly with time and are less influenced by surrounding conditions than plants in any other developmental phase. The growth here studied is thus that occurring during the first two weeks and during the first month, from the seed.

At the beginning of each period, seeds were planted in plunged pots, the same soil being used for all stations, and the pots were furnished with auto-

---

<sup>1</sup> Botanical contribution from the Johns Hopkins University, No. 47.—In the editing of this paper I have been assisted by Mr. F. M. Hildebrandt.—B. E. L.

<sup>2</sup> The manuscript of this paper was received July 1, 1916. This abstract was preprinted, without change, from these types, and was issued as *Physiological Researches Preliminary Abstracts*, vol. 2, no. 4, January, 1917.

irrigators, to prevent the cultures from ever suffering from lack of soil moisture. The influence of rainfall, as it affects soil-moisture, was therefore removed from the main consideration. Temperature, evaporation and sunshine are thus the climatic conditions with which the study mainly deals.

Only two stations are here considered, Oakland (in the mountains of western Maryland) and Easton on the (eastern shore of Chesapeake Bay). Evaporation was measured by means of standardized cylindrical porous-cup atmometers. Daily maximum and minimum temperatures were obtained in the usual manner. Sunshine records are considered to some extent, as are also those of rainfall and soil-moisture.

After about two weeks of growth from the seed, the following growth measurements were recorded: stem height, average number of leaves per plant, average length and width of mature leaves, and average of the products obtained by multiplying length by width for each leaf. After about a month of growth, these measurements were repeated and, also, the average leaf area and the average dry weight of tops per plant were determined.

Apart from the study of various methods for growing and measuring the plants, measuring the climatic conditions and interpreting the data thus obtained, the present studies also yield some very definite indications regarding the interrelations holding between the various climatic features, on the one hand, and the manner and rate of development of the plants, on the other. These results are summarized below.

1. Considering the entire period of observation at each of the two stations here employed (which embraced nearly the entire frostless season at each station), the complex of environmental conditions experienced at the Easton station was much more efficient in producing growth of soy-bean plants than was the corresponding environmental complex experienced at Oakland. The three criteria mainly used in these studies for measuring plant growth—leaf area, stem height and dry yield of tops—all agreed in pointing to this conclusion. For the first two weeks of growth from the seed, the average daily growth increment in terms of leaf-product (the mean of the products obtained by multiplying the length by the breadth of each leaf) was 1.2 for the Easton season, and 0.9 for the Oakland season. The length of the season employed at Easton was 171 days, while the growing season began later and was terminated earlier at Oakland, where the length of season actually employed was 103 days. The total efficiency of the Easton season to produce plant growth, as in these tests, may therefore be regarded as proportional to  $171 \times 1.2$ , or 205.2, and the efficiency of the Oakland season may similarly be taken as proportional to  $103 \times 0.9$ , or 92.7. The total efficiency of the Easton season of observation (its power to produce plant growth) thus appears to have been 2.21 times as great as was the corresponding efficiency of the Oakland season. About one month of the actual frostless season at Easton was not included in the season of

these studies, however, so that the total efficiency of the Easton frostless season for 1914, measured in terms of leaf-product as here used, was about 2.5 times as great as that of the Oakland frostless season.

2. Of the five criteria by which the growth rates of the experimental plants were compared (stem height, total number of leaves, produced, leaf dimensions, leaf surface and dry weight), those of stem height, leaf surface and dry weight exhibited the greatest differences between different culture periods. The rates of growth in terms of leaf surface and in terms of dry weight varied in a similar manner with the same kind of variations in external conditions, while the growth rates measured in terms of stem elongation varied in another way with the same external differences. It thus appears that the rate of elongation of plant stems is influenced by external conditions differently from the rates of development of leaf surface and of dry weight for the same plants. In dealing with the quantitative relations of plant growth to external conditions it is therefore necessary to distinguish clearly between the various kinds of growth and the various criteria that may be employed in their measurement.

3. The rates of growth in stem height were generally more rapid during the first than during the second fortnight of growth from the seed, for both stations. On the other hand, the rates of increase in leaf area (as approximately measured by means of the leaf-product) were generally more rapid during the second fortnight.

4. The growth rates generally showed very evident seasonal marches, by whatever criterion they were measured, increasing during the first part of the season and decreasing in the autumn. These seasonal marches were most apparent for the first two weeks of growth from the seed, and were most clearly shown by the rate of increase in stem height. They correspond, in general trend, to the seasonal marches of the temperature conditions.

5. The seasonal marches of both the growth rates and the temperature values for Oakland are quite markedly different from those for Easton. Both ranges are greater for Easton than for Oakland. The highest temperature values and the highest growth rates occurred at Easton, and the growing season was terminated by killing frost earlier at Oakland than at Easton. Nevertheless, the last two-week period before autumn frost at Oakland exhibited a higher temperature value and higher growth rates than did the last two-week period before frost at Easton. This difference between the magnitudes of the final minimum growth rates observed at the two stations appears to emphasize one of the main differences between a mild, equable, coastal climate and a much more rigorous mountain climate, as these may influence plant growth. In the milder climate of Easton, with its small daily range of temperature, the frostless season is apt to be prolonged until the growth of many plants is much reduced or entirely checked by low temperature. In the mountain climate of Oakland, however, with its large

daily range of temperature and high nocturnal radiation, very low night temperatures and frosts occur earlier in the season, while the day temperatures and the growth rates of many plants are still high. These differences between the two stations, as regards the temperatures and growth rates exhibited at the close of the season (just before autumn frost), are surely intimately associated with the two types of climate here illustrated, and are of undoubted importance in the consideration of plant life in general. Another difference to be noted between the two stations here considered refers to the time of occurrence, within the growing season, of the maxima of temperature and of growth rates. These maxima occurred about a month earlier at Oakland than at Easton—a fact that may be of significance in the comparative seasonal climatology of these stations, at least for the summer of 1914.

6. The mean rate of leaf enlargement (as measured by the leaf-product) and also the mean rate of increase in dry weight, for the four-week periods of growth, followed seasonal marches that showed a secondary influence of the moisture conditions of the surroundings, as well as the primary one exerted by temperature. No apparent relation exists, however, in the data of the present study, between the growth rates, on the one hand, and the data of either rainfall or evaporation, on the other; perhaps because the culture plants were protected from soil drought by auto-irrigation. The general moisture conditions of the surroundings were measured in terms of the ratio of rainfall to evaporation, however, and it is with reference to this ratio that the above-mentioned secondary influence of these conditions becomes apparent. This moisture influence appears to be most clearly shown by the growth rates for periods when the daily mean temperature was high (66° to 76° F.). Apparently it is the moisture conditions of the second half of the four-week period that are here influential. At the end of a month of growth from the seed the mature leaves are larger when the last two weeks of the period have been characterized by a high value of the rainfall-evaporation ratio than when these two weeks have been drier. This is related to the fact that the leaf development of the first month of growth mainly occurs in the latter half of the period.

7. It appears that temperature was clearly the limiting condition (in the usual sense) for growth during the first two weeks, in practically all cases. During the second two weeks of growth, however, with exactly the same environmental conditions, the moisture relation (rainfall-evaporation ratio) appears in many cases to have been the limiting condition for growth, this being especially true, as has been remarked just above, when the temperature was high. It thus appears that if two plants in different stages or phases of their development are exposed to the same fluctuations in environmental conditions, the limiting condition for one plant during a succeeding period may be of an entirely different nature from that for the other. This must

be due to a difference between the *internal* conditions of the plants at different developmental stages. While this principle is so obvious as to appear not to require emphasis, it seems seldom to have been seriously considered in the literature of ecology and physiology. It must be considered wherever standard plants are employed for the comparison of climates

## INTRODUCTION

### THE GENERAL PROBLEM

The dependence of plants upon climatic conditions is almost self-evident, but the quantitative aspect of the relation between plant activities and climate presents an exceedingly complex problem, the solution of which can not be expected for a very long time. Many investigators have attacked this problem, attempting to measure plant production or crop yield in terms of the climatic conditions observed to be present during the growth period. This sort of research has usually resolved itself into attempts to correlate plant growth with one, or at most two, climatic factors—generally with temperature and rainfall, since these are both subject to marked geographical and temporal variations, and since both produce very evident effects upon the manner of growth of plants. The influence of temperature upon plant growth is marked and easily observed. Rainfall affects plant growth mainly in an indirect way, through its influence upon soil moisture. A number of other factors, however, both climatic and non-climatic in character, are also continually exerting influences on plants, and plant growth is an expression of the effects of all these influences combined. Among these other, less frequently mentioned factors are: quality and intensity of sunlight, the evaporating power of the air, wind velocity, presence or absence of parasitic organisms, and many others.

With so many variable factors entering into the equation that may be thought of as expressing the complex set of relations here suggested, no precise correlation between plant growth and any single factor is to be expected. Nevertheless, temperature or rainfall does sometimes act as the most important variable factor, producing the greatest variations in plant growth from year to year or from season to season in any given region or locality, as has been shown by several workers. Surprisingly close agreements were found by Arctowski<sup>3</sup> and by Smith,<sup>4</sup> between crop yield and amount of rainfall, and Merriam<sup>5</sup> found a distinct general correlation between normal temperature conditions and the present distribution of plant and animal

---

<sup>3</sup> Arctowski, Henryk, Studies on climate and crops: corn crops of the United States. Bull. Amer. Geog. Soc. **44**: 745-760. 1912.

<sup>4</sup> Smith, J. Warren, The effect of weather upon the yield of corn. Monthly Weather Rev. **42**: 78-92. 1914

<sup>5</sup> Merriam, C. Hart, Laws of temperature control of the geographic distribution of plants and animals. National Geog. Mag. **6**: 229-238. 1894.

life on the Pacific coast of the United States. It does not appear, however, that such results are to be generally expected. A large number of factors are constantly varying in nature, and many of these are undoubtedly effective to produce variations in the manner and rate of growth of plants. One single factor may be *most* influential, as rainfall in desert regions generally, but very many other conditions are also important for plant growth, and alterations in any of these effective conditions must surely exert some influence on the rates of the physiological processes in plants subjected to such alterations.<sup>5a</sup>

Not only do external conditions about the plants change, but the plants themselves also change as time goes on; they respond differently to the same external influences at different times in their life cycles or in different stages of their development. It follows that plant growth cannot be capable of expression in terms of climatic or other external factors, excepting by means of an exceedingly complex formula, which should involve all of the effective or controlling conditions. We are probably not yet even acquainted with all the factors that influence plants, nor do we know the action of those with which we are acquainted, so that attempts to establish what might be called a complete environmental formula,—representing the total effectiveness of the surroundings to produce growth, maturation of seed, etc., for any given plant form,—must be postponed for a long time.

The rate of growth of any given plant, however, is itself an expression of the sum total of all the effects of all the external conditions as these acted during the period of measurement.<sup>6</sup> Consequently, if it were possible to grow standard plants in different environments, it should be feasible to measure and compare these environments in terms of their capacities to produce growth in the standard plants.

Of course, such a procedure as that here suggested can be of but relatively little value in the interpretation of crop production, etc., unless the environmental conditions may be assorted into several groups which may be separately studied. To study them in this way involves the problem of maintaining certain groups of conditions sensibly alike for different standard plants, while other groups are varied. Thus the soil conditions, taken as a group, may be similar for a number of plant cultures, while the atmospheric conditions may be different. Differences in growth, etc., may then be considered as due to the influence of the atmospheric complex, acting with the internal conditions that make up the nature of the plants used.

---

<sup>5a</sup> While the present paper was in press there appeared the following very important report of a physical study of the relation of plant transpiration to certain environmental conditions:—

Briggs, L. J., and H. L. Shantz, Daily transpiration during the normal growth period and its correlation with the weather. *Jour. Agric. Res.* 7: 155-212. 1916. See also: Kiesselbach, T. A., Transpiration as a factor in crop production. *Nebraska Agric. Exp. Sta. Research Bull.* 6. 1916.

<sup>6</sup> Livingston, B. E., Climatic areas of the United States as related to plant growth. *Proc. Amer. Phil. Soc.* 52: 257-275. 1913. See especially page 258.

This method of study should be valuable, of course, only when it may be assumed that the standard plants were alike at the beginning of the period of measurement. If they were not sensibly alike, interpretation of the observed differences in growth becomes practically impossible, for in such a case the argument is hopelessly complicated by the fact that the different internal conditions initially effective in the various cultures enter into the logical analysis. To interpret the results obtained with plants that were unlike at the beginning of the experiment, an analysis of the internal conditions would first have to be made, and this presents far more difficulties than does the analysis of the relation between external conditions and the rate of growth.

The mode of attack thus suggested was followed in planning the study here to be reported.<sup>7</sup>

#### GENERAL PLAN OF STUDY

*The plants.* To investigate the influence of climatic conditions upon the growth of standard plants, it thus appears desirable to grow cultures of these plants under the different climatic complexes that are to be considered, and to treat all the cultures alike in all other respects. Such like treatment of the cultures cannot be actually attained as yet, but an approach to this is possible. While it is to be remembered that plants vary greatly among themselves, on account of various conditions as yet not well understood, and that they cannot at present be standardized in the same sense as thermometers and many other physical instruments, nevertheless this kind of study may be expected to elucidate some, at least, of the fundamental relations of plant growth to climatic conditions.

The choice of standard plants for such investigations is rendered difficult by several considerations. Since any given plant individual alters with the progress of time and according to its treatment, it is clear that plants that have been subjected to different treatments before the beginning of any comparative test are not to be employed. The standard plants must be considered as integrating instruments and, ideally, should be set at the "zero points" of their scales when the tests begin. This means that the plants employed must be in a stage of their development such that any differences that may have occurred in their past treatment have been registered in growth, internal change, etc., to as slight a degree as possible. Such a stage is presented in the seed; in this dormant phase the organism is but slightly affected by ordinary environmental variations. The seed was therefore chosen as representing the zero point of growth in the study be here presented.

---

<sup>7</sup> A short preliminary paper covering certain phases of this study has already appeared: McLean, Forman T., Relation of climate to plant growth in Maryland. *Monthly Weather Rev.* 43: 65-72. 1915.

The species here employed were soy-bean (*Glycine hispida* Maximov.), Windsor bean (*Vicia faba* L.) maize (*Zea mais* L.) and wheat (*Triticum sativum* L). Only the results obtained with the first of these species, soy-bean, will be dealt with in the present paper. In order that the seeds actually used for comparing the different sets of climatic surroundings might be as nearly alike as possible, they were all of the same strain and of the same crop, for each species employed. Furthermore, each lot was sorted, and all that appeared abnormal for any reason were discarded.

The plants were grown from the seed in cultures in which all environmental conditions except those usually regarded as climatic were controlled as fully as was practicable, and their rates of growth were used as a measure of the effectiveness of the whole group of surrounding conditions. The rate of growth is, of course, the amount of growth accomplished in a given unit of time, and it may be measured in terms of the *amount of plant* produced during the observation period. The quantities measured in this study were the size and weight of the plant produced, beginning with the seed, during periods of about two weeks and of about four weeks after planting. Dividing the result thus obtained by the number of days in the period gives the average daily rate of growth. The average of the rates of growth for several plants was taken as a measure of the comparative effectiveness of the climatic conditions as these tended to produce differences in plant activity at the several stations, during the growth period. These average growth rates were compared to values obtained by instrumental measurements of the climatic factors surrounding the plants during their period of growth.

The cultures were started approximately every two weeks during the growing season, at each of the stations employed, and the growth obtained in each culture was determined after about two weeks and again after about a month.

*Measurement of the climatic conditions.* No attempt was here made to secure a complete evaluation of the climatic conditions that affect plants. Indeed, such an attempt must be quite futile until much more is known about what climatic factors do effect plant growth, and how they act. The regular observations of the U. S. Weather Bureau, for temperature, rainfall and duration of sunshine, with supplemental data bearing on soil moisture and evaporation, were brought together in various ways, for comparison with the plant growth rates for corresponding periods.

Nine coöperative stations of the U. S. Weather Bureau in Maryland were employed in this study. Their geographical locations are shown on the chart of figure 1. Of four stations on the Coastal plain, three (Easton, Princess Anne, and Coleman) are east of Chesapeake bay, and the remaining one (College Park) is much farther inland, near the borderline between the Coastal plain and the Piedmont plateau. Four stations are on the Piedmont plateau, one (Baltimore) at its lower edge near Chesapeake bay, two

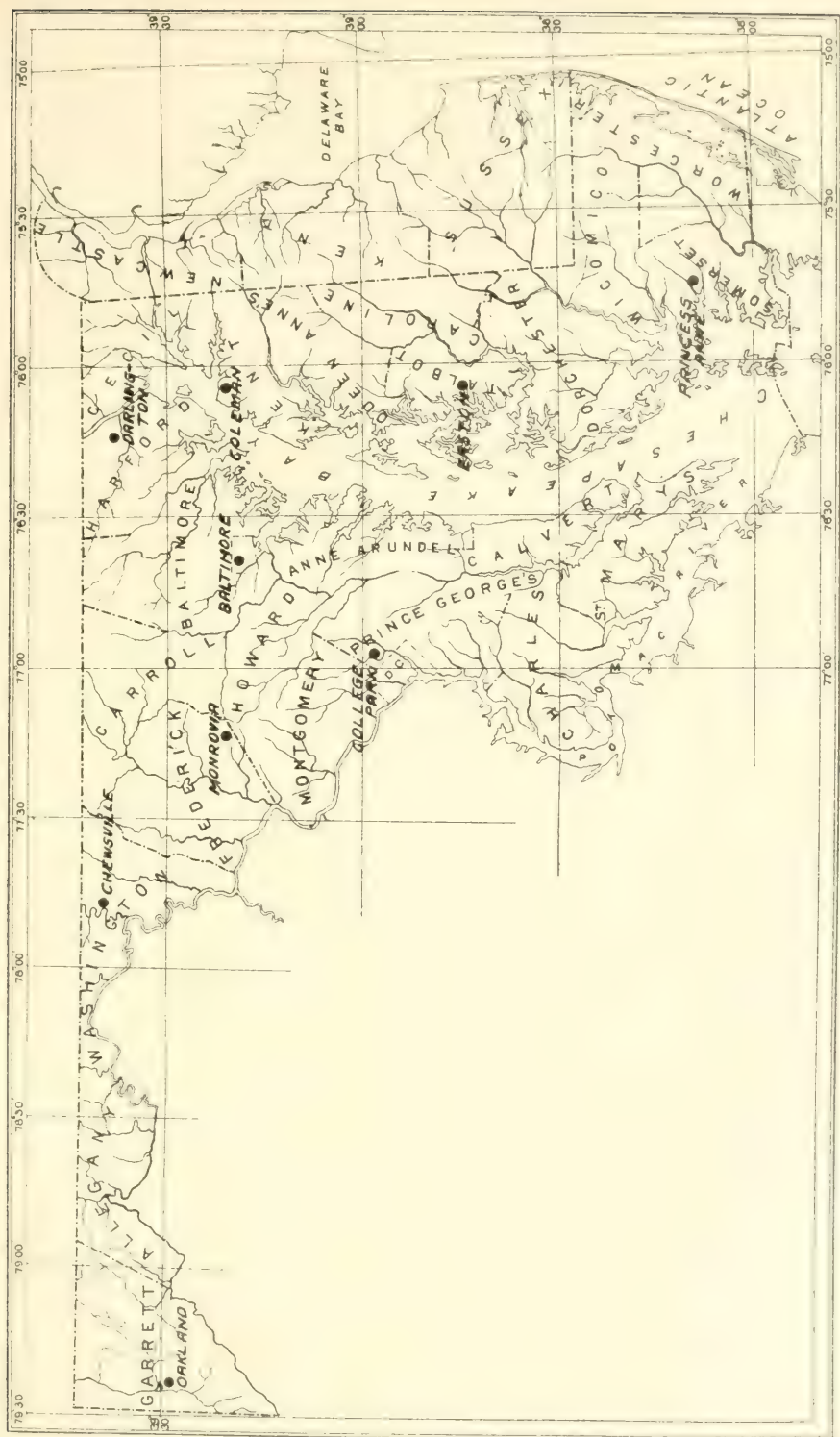


FIG. 1. Chart of Maryland, showing locations of the nine stations employed, only two of which, Oakland and Easton, are considered in the present paper.

(Darlington and Monrovia), in the hilly country north and west of Baltimore, and one (Chewsville) in the Hagerstown valley. The ninth station (Oakland) is on the tableland at the top of the Allegheny plateau. All of these stations excepting Oakland are at comparatively low elevations, less than 1000 feet above sea level. Oakland has an altitude of about 2500 feet.

Without the most cordial support of the coöperative weather observers at the various stations this project could not have been successful, and the writer takes pleasure in here expressing his grateful appreciation of their very generous assistance. It is with much regret that mention must here be made of the death of Mr. J. S. Harris, the observer at Coleman. The other observers who coöperated in this work were: Mr. A. F. Galbreath, of Darlington; Mr. J. H. Lawson of Monrovia; Mr. D. P. Oswald, of Chewsville; President H. J. Patterson, of College Park; Mr. H. Shreve, of Easton; Mr. J. R. Stewart, of Princess Anne; and Mr. R. E. Weber, of Oakland.

Of the nine stations only two, Easton and Oakland, will be considered in the present paper, these being chosen to represent extremes of climatic conditions. Easton is on the flat coastal plain and has the characteristic humid, equable climate of the eastern shore of Chesapeake bay. It has an altitude of only 63 feet above sea level. The climate of Oakland, on the other hand, is typical of the moist but variable climate of the Allegheny plateau. The latter station lies on a gentle south slope, at an altitude of about 2500 feet.

#### ACKNOWLEDGEMENTS

The present studies were carried out under the auspices of the Maryland State Weather Service, and those of the Laboratory of Plant Physiology of the Johns Hopkins University, during the summer of 1914. The study was under the general direction of Prof. B. E. Livingston, to whom the author wishes here to express his great indebtedness for advice and aid, which alone made the work possible. The writer wishes also to acknowledge his indebtedness to Prof. W. B. Clark and to the other members of the Board of Governors of the Maryland State Weather Service, for their esteemed support; to Dr. Oliver L. Fassig, Director of the Maryland Division of the U. S. Weather Bureau, for assistance in securing and compiling the weather data here used and in the selection of the stations at which the work was done; and to his associates, in the general project, Miss A. Hopping, Dr. J. W. Shive and Mr. E. S. Johnston, for coöperation and assistance. He also wishes to express his thanks to Mr. J. Calvert, who most kindly allowed the soil used in this study to be taken from his property, and to Prof. W. T. L. Taliaferro and to Mr. Grover Kinsey, who arranged for and supervised the shipment of the soil to the various stations.

## METHODS AND EXPERIMENTATION.

## THE PLANTS

*The seeds and their treatment.* The soy-bean used in this study was of the variety called "Peking," with small black seeds. The plants are rather small but erect growers. The seed was of pure strain, obtained from the 1913 crop of the Maryland Agricultural Experiment Station. Tests of it showed 98 per cent. of germination, when planted in moist quartz sand, in earthen pots in the greenhouse, at a temperature of about 58° to 71°F., during the early part of March, 1914. The seeds appeared very uniform. All small and all unusually large seeds, as well as any that appeared otherwise abnormal, were discarded.

All the seeds were treated with carbon bisulphide vapor for one week, to destroy insects. Immediately after treatment, on March 27, 1914, the seeds were transferred to paraffined paper cylinders holding a quart, with tight-fitting covers. They were then stored in a laboratory locker, with a rather uniform temperature of about 72°F., until taken into the field for use.

The seeds were planted 2.5 cm. (1 in.) deep, in moist soil in 15 cm. (6 in.) flower pots of the usual conical form, six seeds being planted in each pot. No records were kept of the dates of appearance of the seedlings above the soil at either of the two stations here considered, but such records are available for three of the other stations, Chewsville, Darlington and Monrovia. The average time required for the plants of soy-bean to appear above the ground at these three stations was 5.5 days from the date of planting. In the majority of cases this time was from 4 to 6 days, the extremes recorded being 3 and 11 days. The slow rates of germination occurred during cold periods. The rapidity of germination will not be considered in the discussions that follow; the date of planting appears to furnish a more satisfactory initial point for calculating the growth rate than would that of the appearance of the seedlings above the soil.

*The growth measurements.* Growth may be considered as essentially the process of development toward mature size, and it may be measured in several ways. In this investigation the growth rate of the soy-bean plants was considered from four points of view: (1) the rate of elongation of the plant shoot, (2) the rate of production of leaves, (3) the rate of development of leaves [in terms (a) of linear dimensions, and (b) of superficial area], and (4) the rate of increase in the dry weight of the plant. It is important that the measurements made in securing this sort of data be as rigidly uniform as possible, to render the results comparable, and a regular method and order of measurement were therefore instituted and adhered to throughout the season.

Each station was visited at intervals of approximately two weeks. At the time of the first visit after planting, the height of the stem, and the length

and greatest width of each leaf or leaflet were separately recorded for each plant. The stem height was measured from the soil surface to the base of the terminal bud; the base of the bud was here employed, rather than of the extreme tip, because the length of the bud is affected by the stage of development of the youngest visible leaf, and is therefore variable. The leaves were measured in regular order, from the base of the plant upward, each one being given a serial number in this order in the records. The leaf lengths were measured from the junction of blade with petiole to the tip of the blade. The width was measured at the widest part, and at right angles to the longitudinal axis of the blade. All linear measurements were made to the nearest millimeter.

Approximately four weeks after planting all the measurements just mentioned were repeated, after which the plants were cut off at the level of the soil. The leaves were immediately placed in a photographic printing frame and sun prints were made on photographic paper, from which the extent of the total leaf surface was later obtained, by means of a planimeter. Any plants that appeared to be unusual, because of accidental injury, and those that were markedly smaller or larger than the average were discarded. The plants of each culture were mailed to the laboratory at Baltimore, where they were dried for several days in the greenhouse, and then desiccated in an oven at 100°C., to constant weight. The dry weights were determined to 0.01 gram. These original dry weights included the cotyledons, when present, but these do not properly constitute a part of the growth of the plant after germination, and so the dry weights were afterwards corrected by determining the weight of the cotyledons in each case and subtracting this from the original amount recorded. This correction has been applied to all of the dry weight data employed in the present paper.

As has been indicated, one culture of each of the four species used was started approximately every two weeks, at each station, and each culture was measured as described above. The results of these numerous measurements for soy-bean are summarized in tables I to IV, tables I and III referring to the measurements of the plants when about two weeks old from seed, and tables II and IV referring to the second (and final) measurements of the plants, when they were about four weeks old from seed. These tables are similar in form, so that an explanation of tables I and II, for Oakland, will also serve to describe the manner of presentation of tables III and IV, for Easton.

In table I the first four lines give general data. The different cultures are numbered in chronological order in the first line. The "date of planting" is given in line 2. "Number of plants" (line 3) refers to the number actually used in the measurements and thus excludes abnormal individuals which were discarded.

The data of lines 5 to 13 present a summary of the plant measurements

obtained about two weeks after planting. "Age" (line 5) indicates the length of the period, in days, from the date of planting to the date of observation, which was only approximately two weeks, the variations in this respect being due to the exigencies of the many trips necessary to each of the nine stations that were under observation. In reckoning these ages the date of planting was not included, the period beginning with the day after that date and ending with the date of observation. This age varies from 9 days in the case of culture 1 at Oakland to 17 days for culture 1 at Easton (table III). These differences necessitate that all of the data be ultimately expressed in the form of mean daily rates, or averages, in order that comparison of the different cultures may be possible. In all the plant measurements here given the data are averages per plant, the number of plants from which these averages are derived being given in the third line. Thus, the recorded stem height (line 6) for culture 1 (2.4 cm.) is the average height of the four plants of that culture, being expressed with the same degree of accuracy as in the case of the original measurements. The average daily increase in stem height (line 7) is obtained by dividing the average stem height (line 6) by the corresponding number of days (line 5), in each case. Line 8 shows the average number of full-grown leaves per plant, this number being obtained by summing the number of leaves that had been developed, whether these were still present at the time of measurement or had previously died and fallen. Leaves that were approximately half-grown were considered as half leaves. The purpose of this enumeration is to get an expression of the stage of development of the plants; that is, to show how far the plants had progressed in their life cycle. Thus, a plant with three leaves mature and one half-grown is recorded as more advanced in growth than a plant with only three leaves, but appears as less advanced than a plant with the fourth leaf fully developed. Line 9 gives the average daily increment in the average number of leaves per plant, these data being obtained by dividing each number in line 8 by the corresponding number of days (line 5).

The average leaf dimensions ( $l$  and  $w$ ), given in lines 10 and 11, serve for comparison of the relative sizes of *mature* leaves in the different cultures. The unit of enumeration here employed was not the same as in the case of the number of leaves present (line 8). For the data of line 8 all leaves, whether mature or only partly grown, were considered, and the whole leaf, whether simple or compound, was taken as a unit; for the leaf dimensions  $l$  and  $w$ , on the other hand, only mature leaves were measured (or those very nearly mature, for small plants), and here the unit is a leaf or a leaflet, as the case may be. It is of no serious moment for the present purpose, whether the unit of surface is a leaflet of a compound leaf (as the secondary, alternate leaves of soy-bean) or a simple leaf (as each of the initial pair of opposite leaves). The average "leaf-product,"  $P$  (line 12), is the average per plant of the sum of the products of length multiplied by width for *all*

leaves, whether young or mature. These average leaf-products serve for general comparison of the relative leaf areas of the plants in the different cultures. The average daily increase in leaf-product (line 13) is obtained by dividing the average leaf-product (line 12) by the corresponding number of days (line 5). The derivation of the soil moisture percentage (line 14) will be fully explained in connection with the discussion of soil environment, so that it will suffice to state here that each figure given is the average of the soil moisture data obtained for the culture in question during the period covered by the corresponding plant measurements.

The data given in table II, also for Oakland, are the final measurements of the plants at the time of harvest, when they were approximately four weeks old. The data given in lines 1 to 13 and in line 19 correspond to the similar data of table I, lines 1 to 14, and need no further comment here. Lines 14 to 18, however, present data not considered in table I. "Leaf area" (line 14) is the average total area per plant of either leaf surface, the lower or the upper, but not of the total leaf surface, which would be double the value given. This leaf area ( $A$ ) was obtained by means of a planimeter, from photographic prints of the leaves, as has been mentioned. The average daily increment of leaf area (line 15) is obtained by dividing each number in line 14 by the corresponding number of days (line 5). The average dry weight (line 16) is the average weight per plant of only the top portion, excluding the parts beneath the soil surface, as has also been stated. The average daily increment in dry weight (line 17) is the quotient of the average dry weight (line 16) divided by the corresponding age (line 5). The ratio of the average leaf-product to the average leaf area  $\left(\frac{P}{A}\right)$  is given in line 18. This will receive attention later.

*Appearance of plants.* Tables I to IV give the measurements of the plants grown in the different cultures at the two stations, but they give no information about the manner of development of the plants or about the possible variable influences that may have affected them and that were neither measured nor controlled. It is desirable to consider here the general thrift and observed development of the plants, as well as some of the variable non-climatic factors that may have influenced the cultures.

At the time of the first observations, about two weeks after planting, the cotyledons, which were still green and in healthy condition, were always still attached to the young seedlings. The leaves were also bright green and appeared thrifty. During the first two weeks, then, the cotyledons being still attached, it may be supposed that the seedlings were subsisting upon the stored nutriment of the seeds, at least in part.

At the time of the final observations, on the other hand, these conditions were different, but variable. At Oakland where the plants had grown rather slowly, the cotyledons were still green, still attached, and probably

TABLE I  
*Numerical data for soy-bean cultures approximately two weeks old from date of planting; Oakland, Md., 1914*

	1	2	3	4	5	6	7	8	9	Average
(1) Number of culture.....	1	2	3	4	5	6	6	6	4	5
(2) Date of planting.....	May 26	June 4	June 18	July 2	July 15	July 30	Aug. 13	Aug. 26	Sept. 11	Sept. 24
(3) Number of plants.....	4	5	5	6	5	6	6	6	4	5
(4) Date of observation.....	June 4	June 18	July 2	July 15	July 30	Aug. 13	Aug. 26	Sept. 11	Sept. 24	Sept. 24
(5) Age, days.....	9	14	14	13	15	14	13	16	13	13
(6) Average stem height ( <i>H</i> ), <i>cm.</i> .....	2.4	4.8	6.1	3.9	5.1	4.9	4.7	3.0	1.9	4.1
(7) Average daily increase in stem height, <i>cm.</i> .....	0.27	0.34	0.44	0.30	0.34	0.35	0.36	0.19	0.15	0.30
(8) Average number of leaves.....	1.0	2.0	2.0	2.0	2.4	2.0	2.0	1.5	0.0	1.7
(9) Average daily increase in number of leaves.....	0.11	0.14	0.14	0.15	0.16	0.14	0.15	0.94	0.0	0.14
(10) Average length of mature leaves ( <i>L</i> ), <i>cm.</i> .....	1.3	2.9	3.1	2.6	3.1	2.9	2.8	2.6	0.0	2.4
(11) Average width of mature leaves ( <i>w</i> ), <i>cm.</i> .....		2.2	2.2	2.0	2.4	2.3	2.1	1.7	0.0	2.1
(12) Average leaf-product ( <i>P</i> ) for all leaves.....		12.8	13.7	10.8	15.0	12.2	11.9	8.4		12.1
(13) Average daily increase in leaf-product.....		0.91	0.98	0.83	1.00	0.87	0.92	0.52		0.96
(14) Soil moisture, per cent. of dry weight.....		13.1	14.7	17.9	18.3	15.3	17.9	18.5	16.4	16.5

TABLE II  
*Numerical data for soy-bean cultures approximately four weeks old from date of planting; Oakland, Md., 1914*

	1	2	3	4	5	6	7	8	Average
(1) Culture number	May 26	June 4	June 18	July 2	July 15	July 30	Aug. 13	Aug. 26	
(2) Date of planting	4	5	5	6	5	6	6	6	5
(3) Number of plants	June 18	July 2	July 15	July 30	Aug. 13	Aug. 26	Sept. 11	Sept. 24	
(4) Date of observation	23	28	27	28	29	27	29	29	28
(5) Age, <i>days</i>	4.8	7.0	8.1	6.1	6.6	7.1	5.9	4.2	6.2
(6) Average stem height ( <i>H</i> ), <i>cms.</i>									
(7) Average daily increase in stem height, <i>cm.</i>	0.209	0.250	0.300	0.218	0.228	0.263	0.203	0.145	0.227
(8) Average number of leaves	2.75	3.40	3.20	3.50	3.80	3.50	3.00	2.80	3.24
(9) Average daily increase in number of leaves	0.12	0.12	0.12	0.13	0.13	0.13	0.10	0.10	0.12
(10) Average length of mature leaves ( <i>l</i> ), <i>cm.</i>	2.5	2.8	3.1	2.8	2.9	3.4	3.1	2.7	2.9
(11) Average width of mature leaves ( <i>w</i> ), <i>cm.</i>	1.8	2.1	2.4	2.1	2.1	2.4	2.2	1.9	2.1
(12) Average leaf-product ( <i>P</i> ) for <i>all</i> leaves	16.8	32.0	38.2	34.8	37.5	40.1	33.2	21.0	31.7
(13) Average daily increase in leaf-product	0.73	1.14	1.41	1.24	1.29	1.49	1.15	0.90	1.17
(14) Average leaf area ( <i>A</i> ), <i>sq. cm.</i>	11.6	22.9	29.9	25.6	28.0	29.8	25.5	16.5	11.2
(15) Average daily increase in leaf area, <i>sq. cm.</i>	0.50	0.82	1.11	0.91	0.97	1.10	0.88	0.57	0.86
(16) Average dry weight ( <i>W</i> ), <i>grams.</i>	0.075	0.150	0.160	0.134	0.142	0.125	0.123	0.069	0.122
(17) Average daily increase in dry weight, <i>grams.</i>	0.0033	0.0054	0.0039	0.0048	0.0049	0.0046	0.0042	0.0024	0.0044
(18) Ratio of leaf-product to area ( $\frac{P}{A}$ )	1.44	1.39	1.28	1.36	1.34	1.35	1.30	1.27	1.34
(19) Soil moisture, <i>per cent. of dry weight</i>	12.0	14.5	17.2	17.5	16.0	15.8	16.9	14.8	15.6

TABLE III  
*Numerical data for soy-bean cultures approximately two weeks old from date of planting; Easton, Md., 1914*

(1) Culture number	1	2	3	4	5	6	7	8	9	10	11	12	Average
(2) Date of planting	May 8	May 25	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Aug. 31	Sept. 14	Sept. 28	Oct. 11	
(3) Number of plants	6	4	5	6	4	5	5	5	5	6	6	3	5
(4) Date of observation	May 25	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Aug. 31	Sept. 14	Sept. 28	Oct. 11	Oct. 26	
(5) Age, <i>days</i>	17	14	14	14	14	14	14	14	14	14	13	15	14
(6) Average stem height ( <i>H</i> ), <i>cm.</i>	2.7	4.4	5.6	6.6	7.0	7.3	6.6	5.9	4.0	2.3	3.0	1.8	4.8
(7) Average daily increase in stem height, <i>cm.</i>	0.16	0.31	0.40	0.47	0.50	0.52	0.47	0.42	0.29	0.16	0.23	0.12	0.34
(8) Average number of leaves	2.0	2.0	2.0	2.5	2.8	3.0	3.0	2.5	2.0	1.0	1.0	0.0	2.2
(9) Average daily increase in number of leaves	0.12	0.14	0.14	0.18	0.20	0.21	0.21	0.18	0.14	0.072	0.072		0.16
(10) Average length of mature leaves ( <i>l</i> ), <i>cm.</i>	3.0	3.1	3.4	3.3	3.4	3.4	3.4	3.7	2.6	1.9			3.1
(11) Average width of mature leaves ( <i>w</i> ), <i>cm.</i>	2.3	2.4	2.5	2.6	2.7	2.4	2.6	2.8	1.9	1.4			2.4
(12) Average leaf-product ( <i>P</i> ) for all leaves	13.6	15.3	17.5	15.6	23.8	20.4	25.5	20.2	10.1	5.4	0	0	16.7
(13) Average daily increase in leaf-product	0.80	1.09	1.25	1.11	1.70	1.46	1.82	1.44	0.72	0.39	0.0		1.18
(14) Soil moisture, <i>per cent. of dry weight</i>	10.1	12.7	13.3	14.4	13.5	15.3	12.0	14.6	13.5	14.6	14.4	15.4	13.7

TABLE IV  
*Numerical data for soy-bean cultures approximately four weeks old from date of planting: Easton, Md., 1914*

(1) Culture number	1	2	3	4	5	6	7	8	9	10	11	12	Aver., age
(2) Date of planting	May 8	May 25	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Aug. 31	Sept. 14	Sept. 28	Oct. 11	.....
(3) Number of plants	6	4	5	6	4	5	5	5	5	6	6	3	5
(4) Date of observation	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Aug. 31	Sept. 14	Sept. 28	Oct. 11	Oct. 26	Nov. 6	.....
(5) Age, <i>days</i>	31	28	28	28	28	28	28	28	28	27	28	26	28
(6) Average stem height ( <i>H</i> ), <i>cm.</i>	4.7	6.4	8.2	9.1	9.1	10.0	9.3	7.3	5.1	4.2	5.1	2.3	6.7
(7) Average daily increase in stem height, <i>cm.</i>	0.152	0.229	0.293	0.325	0.325	0.357	0.332	0.261	0.182	0.155	0.182	0.088	0.240
(8) Average number of leaves	3.3	3.0	4.0	4.0	3.8	4.0	4.0	3.0	2.8	2.7	2.5	1.0	3.2
(9) Average daily increase in number of leaves	0.11	0.11	0.14	0.14	0.14	0.14	0.14	0.11	0.10	0.10	0.089	0.039	0.11
(10) Average length of mature leaves ( <i>l</i> ), <i>cm.</i>	2.9	3.0	3.0	2.9	3.3	3.2	3.2	3.3	2.9	3.0	2.8	1.5	2.9
(11) Average width of mature leaves ( <i>w</i> ), <i>cm.</i>	2.2	2.3	2.2	2.3	2.5	2.4	2.3	2.5	2.1	2.2	2.1	1.2	2.2
(12) Average leaf-product ( <i>P</i> ), for all leaves	36.7	34.5	50.0	41.3	42.9	55.5	56.8	42.8	28.9	26.7	25.8	3.7	37.1
(13) Average daily increase in leaf-product	1.18	1.23	1.79	1.48	1.53	1.98	2.03	1.53	1.03	0.99	0.92	0.13	1.32
(14) Average leaf area ( <i>A</i> ), <i>sq. cm.</i>	28.0	25.7	37.7	30.8	34.5	41.3	41.1	32.2	22.5	21.0	20.0	2.6	28.1
(15) Average daily increase in leaf area, <i>sq. cm.</i>	0.90	0.92	1.35	1.10	1.23	1.47	1.47	1.15	0.80	0.78	0.71	1.00	1.07
(16) Average dry weight ( <i>W</i> ), <i>grams</i>	0.155	0.135	0.206	0.178	0.198	0.214	0.212	0.140	0.110	0.07	0.073	0.016	0.142
(17) Average daily increase in dry weight, <i>grams</i>	0.0050	0.0048	0.0074	0.0064	0.0071	0.0076	0.0076	0.0050	0.0039	0.0026	0.0026	0.0006	0.0051
(18) Ratio of leaf-product to area ( $\frac{P}{A}$ )	1.31	1.35	1.33	1.34	1.24	1.34	1.34	1.33	1.28	1.27	1.29	1.42	1.32
(19) Soil moisture <i>per cent. of dry weight</i>	10.1	12.4	14.2	13.6	13.4	14.4	12.4	14.6	12.7	13.9	14.2	15.4	13.5

still furnishing material to the growing plants. At Easton, however, this state of affairs was the exception; it was encountered only toward the end of the season, when the plants had grown slowly. In most of the Easton cultures the majority of the plants had either lost their cotyledons, or the latter had turned yellow and were about to fall. Thus, these Easton plants were probably generally independent of the stored food supply of the seeds, at the time of the final observation. Furthermore, these plants were not as healthy in appearance as those in the corresponding stage at Oakland, their leaves being yellowish in color.

It is possible that this unhealthy appearance of the largest and most mature plants at Easton may have been due to the absence of nutrifying bacteria on their roots. The soil used in these experiments had previously supported, among other plants, a wild vetch and is thus to be considered as provided with at least some nodule-forming bacteria, but these may not have been of the right kind for soy-bean. Root nodules appeared in the cultures of Windsor bean but not in those of soy-bean. The absence of root nodules in case of soy-bean, while it almost surely affected the growth rates of the plants, was not a variable factor, as this condition held throughout all of the cultures, and hence it should not interfere with the value of these cultures in comparing the effects of climatic conditions. No other apparently important non-climatic variable factors, which might seriously have affected the growth rates, were noted in the case of these soy-bean cultures.

## THE ENVIRONMENTAL CONDITIONS

### EXPOSURE OF INSTRUMENTS AND PLANTS

*Local and instrumental exposure.* The presence of trees or other objects in the near vicinity of the growing plants or of the climatological instruments may seriously influence such environmental conditions as the evaporating power of the air, the intensity of sunshine, wind movement, etc., for the particular exposure in question. It is therefore desirable to have the plants and the instruments located near together, and exposed as similarly as possible so that the growth of the plants and the readings of the instruments may refer to similar atmospheric conditions. It is also desirable that the culture and instrument locations be as much in the open as possible, in order that they may represent the general conditions of the region as a whole and in order that the data obtained at the different stations may be studied in connection with the regional climatic conditions.

The weather stations for both Oakland and Easton are situated in the open country. The Oakland station is on a south slope, about 1.6 km. (1 mi.) east of the town of Oakland, and near a public road, which is some-

what dusty in dry weather. The general nature of the exposure of the plant cultures and instruments at Oakland is shown in figure 2. The cultures, the rain gage and the thermometer shelter were situated near together, about 12 m. (nearly 40 ft.) west of the greenhouses of H. Weber's Sons, and were fully exposed on all sides. The greenhouses, being only about 4.5 m. (15 ft.) high, did not produce serious wind obstruction, and they did not influence the light conditions appreciably.

The Easton station was north of the residence of the observer, Mr. Henry Shreve, the general surroundings of the various instruments being shown in figure 3. The plant cultures were 1.2 m. (4 ft.) north of the rain gage and 18.3 m. (60 ft.) northwest of the dwelling. This position was protected



FIG. 2. Culture enclosure and its general surroundings, at Oakland.

on the south, southwest and west by large apple trees, about 15 m. (nearly 50 ft.) distant, and by a low shed about 10 m. (33 ft.) distant on the north. The exposure is open toward the east. The crowns of the trees are too high to obstruct wind movement near the ground in a serious manner, but the plant cultures were shaded by the trees from about 3:30 until sunset, in June, and from 1 o'clock in September. The thermometer shelter stands under a tree west of the culture location and is about 6 m. (20 ft.) north of the house. Thus, all of the instruments at Easton as well as the plants, were somewhat protected, but not entirely screened, from strong winds from the north, west and south, and the plants were shaded in the late afternoon.

The most pronounced difference between the exposure at Oakland and

that at Easton lies in the amount of sunlight received. The Oakland plot was exposed to full sunlight throughout the day, while the Easton plot was shaded in the late afternoon.

The climatological instruments (rain gage and thermometers) are of the standard pattern employed by the U. S. Weather Bureau, and have the standard exposure. The thermometers are in the usual shelter, 1.5 m. (5 ft.) above the ground, and thus do not experience all the temperature changes to which are subjected young growing plants near the soil surface. The air temperature around such plants is often greatly influenced by radiation



FIG. 3. Culture enclosure (partly shown to the left of the rain gage) and its general surroundings, at Easton.

from the soil, while thermometers with the standard exposure of the U. S. Weather Bureau are much less influenced in this way. The warming of the earth by isolation, and the rapid cooling on clear nights, by radiation into the atmosphere, subject low plants to extremes of temperature not usually recorded by the thermometers. It seems fair to suppose, however, that there is probably a fairly constant relation between the average daily marches of temperature for these two heights above the soil, in localities where the soils are similar in physical character, color and moisture-content, as is the case for the two stations here considered. The soils surrounding the plant cultures at both Oakland and Easton are rather heavy loams with brown

top soil. They are somewhat strongly retentive of moisture, and are thus apt to be rather cold as compared to the air above them. It thus appears that the temperature conditions to which the culture plants were exposed at Easton and at Oakland may be safely compared, for the present purpose, by means of the thermometer readings obtained from the instruments in the elevated shelters.

*Plant exposure.* The plant cultures were protected from accidental injury by enclosing the cultures at each station in a wire-covered frame. The

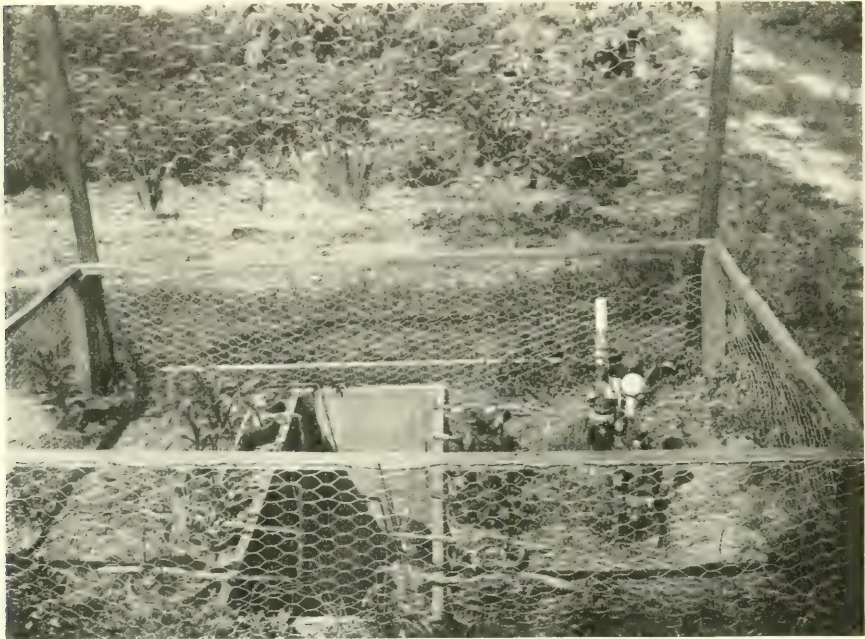


FIG. 4. Interior view of culture enclosure, with top raised, showing irrigator pit, plunged pots and atmometer. (The spherical atmometers were being subjected to preliminary tests and their readings are not considered in the discussion.)

form and arrangement of this is shown in figure 4. It was rectangular, 1.22 m. (4 ft.) from north to south, 1.83 m. (6 ft.) from east to west, 45 cm. (18 in.) high, and was surrounded with galvanized iron wire netting having meshes 2.5 cm. (1 in.) in diameter. This cage was provided with a removable top consisting of a frame covered with wire netting, with meshes 5 cm. (2 in.) in diameter. A pit 61 cm. (2 ft.) deep, 1.22 m. (4 ft.) long from north to south and 41 cm. (16 in.) wide, was dug across the center of the enclosure, and was walled up with boards. The plant cultures, in ordinary flower pots, were arranged in two rows of six each just outside of the pit, one row

along each of its longer sides. The pots were plunged to such a depth that the soil surface of each pot was about level with that of the surrounding soil.

As already stated, a new culture for each of the four plant species was started at each station approximately once every two weeks, and each culture was continued for four weeks. Thus the culture periods overlapped, and there were regularly two sets, of four pots each, at each station. In addition to these, a third set of four pots, without plants, was constantly maintained at each station. The twelve pots, which were thus always present after the third visit to each station, were arranged in two rows running from north to south, a row of six on either side of the pit, and were so placed as to avoid as much as possible having the plants of the different cultures shade each other. The successive sets of four cultures each, were placed, two pots on each side of the pit, beginning with the north end, so that the younger plants were never directly north of the older ones. Also the cultures of the different species of the same age were arranged so that the more vigorous growers would not shade the slower-growing forms. Thus, Windsor bean was placed north of the corresponding soy-bean culture on the east side of the pit, and maize was placed north of the corresponding wheat culture on the west side.

*The soil conditions.* The same character of soil was used in all of the plant cultures. It was a rather light soil obtained from an untilled field near College Park, Md., and was of the soil type classified as Norfolk sand by Bonsteel.<sup>8</sup> Its water-retaining power was found to be 43 per cent., on the basis of dry weight, by the Hilgard<sup>9</sup> method, which employs a one-centimeter soil column. The top-soil was removed from a small area to a depth of 15 cm. (6 in.), and the soil thus obtained was thoroughly mixed and sifted. It was then placed in cloth sacks and shipped to the various stations, where it was stored in air-dry condition, in covered, water-tight, galvanized iron cylinders, until needed for use in the cultures.

The soil containers for the cultures were ordinary porous clay flower pots, in form like the frustrum of a cone, being smaller at the bottom. Their inside dimensions were: top diameter, 15 cm.; bottom diameter, 9.5 cm.; height, 16 cm. The capacity of each pot was thus approximately 1980 cc. of soil, when level full. However, in filling the pots, two auto-irrigator cups (to be considered below), each occupying about 72 cc., of volume, were also placed in each pot, and the soil was compacted so that it stood about 1 cm. below the top of the pot. The actual volume of soil in the pots when in use was approximately 1645 cc.

In order to secure uniform soil conditions in the cultures, it was necessary not only to have soil of similar character for all cultures, but also to bring

<sup>8</sup> Bonsteel, Jay A., *The soils of Prince George's County*. Publication of the Maryland Geological Survey. Baltimore, 1911.

<sup>9</sup> Hilgard, E. W., *Soils, their formation, properties and composition*. New York, 1911. Page 209.

it into the same physical condition, so that it would retain its structure during the growth period of the plants and so that all cultures of the same age should have practically the same soil conditions. The very desirable condition of loose tilth could not be maintained in these cultures; since they were freely exposed to the weather and were visited only once in a fortnight, every rain must pack loose soil more or less, and heavy rains would saturate it completely. Therefore, to put the soil into a state of aggregation to be least altered by varying weather conditions, the soil was saturated with water immediately after it was put into the pots. This was accomplished by plunging the pots of soil into a bucket of water, and allowing them to remain submerged until air bubbles ceased to rise. The pots were then set in position in the enclosures and allowed to drain. After the soil had settled, its surface was found to be one centimeter below the top of the pot, the soil mass having been compacted from 1736 to 1646 cc., or approximately 5.5 per cent. of its loose volume.

The soil moisture in the cultures was maintained by means of Livingston auto-irrigators.<sup>10</sup> This device, as here used, consisted of two cylindrical porous clay cups (of the regular form supplied by the *Plant World*) 15 cm. long and 2.5 cm. in diameter. These were connected with each other and with the water reservoir by glass tubes in the form of an inverted J. The cups were placed vertically in the pot, their rubber-stoppered tops level with the soil surface, and were so arranged as to supply water to the soil against a pressure of 35 cm., or more, of water column. By this arrangement water is withdrawn from the porous cups by the capillary attraction of the water films in the soil about them. The difference between the pressure of the water in the cups and that of the soil films was adjusted in these experiments by placing the 1-gallon (nearly 4-liter) water reservoirs in the pits above described, 60 cm. below the surface of the soil, so that the water level in the reservoir, when the latter was full, was 35 cm. (14 in.) below the soil surface. The moisture content of the soil in the pots was thus maintained so that it was never less than about 10 to 13 per cent. (on the basis of dry weight), in which condition this particular soil was rather too wet than too dry for the best growth of the plants here studied. The soil was often moistened by rains, which sometimes increased the moisture content up to its maximum water-retaining power for the 15-cm. soil columns in the pots (approximately 23 per cent.).

To prevent the movement of water and dissolved salts through the sides of the pot, between the culture soil and that surrounding the pot, the pots were painted on the outside. This coating proved inefficient, however, as the paint soon peeled off. The pots were later surrounded with strips

<sup>10</sup> Livingston, B. E., A method for controlling plant moisture. *Plant World* 11: 39-40. 1908.

Hawkins, Lon A., The porous clay cup for automatic watering of plants. *Plant World* 13: 220-227. 1910.

Transeau, E. N., Apparatus for the study of comparative transpiration. *Bot. Gaz.* 52: 54-60. 1911.

of oilcloth, which appeared to be more satisfactory. Whatever water movement occurred through the pots, however, was probably in the direction from the culture to the outside soil, as the soil in the pots was at all times more nearly saturated than was the soil around them. Thus there is little probability that the soil solution in the pots became greatly modified by the entrance of soil water from without.

After preparing the pots and arranging the watering devices as described above, the pots were then allowed to remain fallow for about two weeks before planting. Thus the soil was fully drained out after the preliminary saturation, and had settled into a condition somewhat nearly approaching that of structure-equilibrium,\* before the seeds were planted. Care was taken to space the seeds uniformly, and to place them about equally distant from the auto-irrigator cups and from the sides of the pots, so that all should have, as nearly as possible under the conditions of the experiments, the same soil moisture conditions.

At the time of planting, and at each fortnightly visit thereafter as long as each culture was continued, a soil sample was taken from each pot for the purpose of soil moisture determination. The method used was that described by Brown.<sup>11</sup> A small cylinder of soil containing about 22.5 cc. (about 30 grams, dry weight) was removed by means of a brass tube (cork borer), which was thrust into the soil vertically at a point midway between the two auto-irrigator cups, and about 3.75 cm. (1.5 in.) from each cup. Each soil sample thus taken represented a vertical section of the soil mass, of uniform diameter and extending from the upper surface to the bottom of the pot. The soil samples thus obtained were immediately transferred to heavily paraffined paper-pulp containers (which were serially numbered), and sealed in these with a paraffin seal. They were then sent, in paste-board mailing-tubes, to Baltimore, where the moisture content was determined. Previous to the beginning of this experiment, the type of paraffined container here used (which is on the market for milk, etc.) was tested, as to its permeability to water vapor and it was found that the greatest loss from moist soil left sealed in such vessels for an entire week was not greater than about 0.1 per cent. of its dry weight. None of the field samples were ever left in the containers for a longer period than this, so that errors in the soil moisture determinations due to leakage may be regarded as negligible. For lightness in transportation and for general ease in handling, these paper containers were found very satisfactory. Larger ones of the same kind were employed for storing the stock of seeds.

When the plants were removed from a pot (about six weeks after that pot was filled) the soil was discarded, and fresh soil from the stored supply was always used in refilling.

<sup>11</sup> Brown, W. H., The relation of evaporation to the water content of the soil at the time of wilting. *Plant World* 15: 121-134. 1912.

TABLE V  
*Determinations of soil moisture, in percentage, on the basis of the dry weight of soil, for the pot cultures at Oakland*

DATE	CULTURE NO.	SOY-BEAN	WINDSOR BEAN	MAIZE	WHEAT	CULTURE NO.	SOY-BEAN	WINDSOR BEAN	MAIZE	WHEAT	CULTURE NO.	SOY-BEAN	WINDSOR BEAN	MAIZE	WHEAT	AVERAGE	EXTREME DEVIATION FROM AVERAGE	
																	plus	minus
May 22.....	1		11.5	7.8	7.7											9.0	2.5	1.3
June 4.....	1																	
June 18.....	1	12.0		11.5	12.2	11.4										12.2	1.4	1.6
July 2.....	4	15.8	17.4	17.7	18.6	15.7					3	13.7	12.0	10.7	13.2	16.5	2.1	2.2
July 15.....	4	20.0	19.9	24.7	19.5	21.3					3	15.6	16.7	15.1	17.1	19.8	3.9	2.8
July 30.....	4	14.9	14.0	15.1	14.6	16.0					6	18.8	19.4	19.1	17.7	15.8	2.2	0.9
August 13.....	7	18.3	18.4	17.4	20.8	17.2					6	15.3	17.2	16.1	18.0	17.1	3.7	2.4
August 26.....	7	17.4	15.9	18.5	18.7	14.7					6	16.2	17.6	16.3	18.2	18.2	2.2	2.3
September 11.....	7	16.4	16.6	18.1	16.4	17.3					9	19.4	20.3	18.6	16.5	17.6	2.7	1.2
September 24.....	10			15.4		13.4					9	13.4	14.5	14.8	15.5	14.3	1.4	1.2
October 9.....	10					16.4					9	14.6	14.4	13.5	15.4	14.9	1.5	1.5
																Mean for the season	16.3	



The results of the soil moisture determinations at Oakland and at Easton are shown in tables V and VI. These are given in detail, for all four species of each culture, to show the variations. The average of all determinations for each day of observation, together with the extreme deviations from the average, are given in the last column. In the cases where no data are given the auto-irrigating device was accidentally broken, and consequently the soil dried out.

In these tables, each date given in the first column applies throughout the line in which it stands. Thus, on July 15, the second sample was obtained from culture 4, the first from culture 5, and the third from culture 3. The kind of plant grown, or to be grown, in the pots is shown above, at the head of each column.

The soil moisture contents of samples taken from different pots at the same station and on the same date are fairly consistent, as is shown by the column of extreme deviations, which generally show values between 1 and 4, approximately, or from about 7 to 25 per cent. of the average soil moisture percentage. The values of the average soil moisture contents are quite variable, however; these averages range in value from 9.0 to 19.8 for Oakland (table V), and from 11.1 to 15.7 for Easton (table VI). These variations seem probably to have been due to differences in the climatic features that affect soil moisture, namely rainfall and evaporation. The irrigators simply kept the moisture content of the soil from becoming lower than about 10 per cent.

#### GENERAL MOISTURE CONDITIONS AND THEIR MEASUREMENT

The water-supplying and water-withdrawing influences exerted by the atmospheric surroundings of ordinary terrestrial plants are to be grouped under two headings; precipitation and evaporation. The first of these (precipitation or rainfall) exerts its main influence upon ordinary plants indirectly, by increasing soil moisture and, consequently, the facility with which the soil may supply water to the plant roots. Precipitation was measured in the usual manner; the amount of moisture intercepted by the funnel of a standard U. S. Weather Bureau rain gage was measured each evening at sunset, and the result was computed as depth of rainfall in inches.

In the present study interest in the environmental moisture conditions centers mainly about the influence exerted by the surroundings to alter the possible rate of water supply or the actual rate of water loss from plants. For ordinary terrestrial plants, which absorb practically all of their water from the soil in which they are rooted, the water supplying capacity of the surroundings is mainly determined by the resistance offered by the soil to water-absorption by plant roots.<sup>12</sup> This resistance varies with the character and

<sup>12</sup> For a more thorough discussion of this general topic, and for accounts of applications of this principle, see: Livingston, B. E., and Hawkins, Lon A., The water-relation between plant and soil. Carnegie Inst. Wash. Pub. 204: 3-48. 1915. Pulling, H. E., and Livingston, B. E., The water-supplying power of the soil as indicated by osmometers. *Ibid.* 204: 49-84. 1915.

structure of the soil and with its moisture content. Since soil of the same character and structure was employed in all of these experiments, the soil moisture content of these cultures may be considered as an approximate index of the power of the soil to supply water to plant roots.

The environmental conditions most influential in determining the rate of water-loss from plants are the evaporating power of the air and the intensity or heat-equivalent of the absorbed radiant-energy, received from the sun.<sup>13</sup> The first of these was measured by atmometers, and the measurements will here be considered as indicating the degree of the tendency of the environment to promote evaporation from the plants. Radiant energy was not measured in this study, though a consideration of the available sunshine data will be presented farther on.

The influence of the evaporating power of the air upon plants is twofold. It may affect the water supply of the plants indirectly, by reducing the soil moisture, and it withdraws water from them directly, by evaporation (transpiration). The evaporating power of the air was measured in these studies by means of cylindrical porous cup atmometers<sup>14</sup> located inside the culture enclosures, 45 cm. (18 in.) from the east end and 30 cm. (1 ft.) from the north side (fig. 4). The atmometer cups were provided with rain-correcting mercury valves, and were mounted upon reservoir bottles of 500 cc. capacity. The reservoirs were placed upright in the soil, and at such a depth that the centers of the atmometer cups were from 28 to 32 cm. above the soil surface. These atmometers were refilled with distilled water and their water-loss was recorded at every fortnightly visit to each station. After every reading each atmometer was replaced by another that had just been standardized, and the used cup was returned to Baltimore, where it was re-standardized. All atmometer readings were reduced to terms of the Livingston cylindrical standard, and they should thus be directly comparable with other measurements on the same basis. The spherical cups shown in figure 4 were employed only for preliminary tests of various makes of these instruments. They were not yet perfected at the time this work was carried out.

The effects of rainfall and evaporation upon soil moisture in the cultures here considered were much reduced by the use of the auto-irrigators, which gave, as has been stated, a minimum soil-moisture content of approximately 10 to 13 per cent., on the basis of dry weight. The soil always appeared dark-colored and moist on the surface, and felt damp to the touch. Rain

<sup>13</sup> Livingston, B. E., Light intensity and transpiration. *Bot. Gaz.* **52**: 417-438. 1911.

<sup>14</sup> Livingston, B. E., A rotating table for standardizing porous cup atmometers. *Plant World* **15**: 157-162. 1912. Other references are there given.

———, Atmometry and the porous cup atmometer. *Plant World* **18**: 21-30, 51-74, 95-111, 143-149. 1915.

Shive, J. W., An improved non-absorbing porous cup atmometer. *Plant World* **16**: 7-10. 1915.

Johnston, E. S., and B. E. Livingston, Measurement of evaporation rates for short time intervals. *Plant World* **19**: 136-140. 1916.

thus influenced the soil moisture content of the cultures only by increasing an already abundant supply of water. The general character of the effects of variations in precipitation and in the evaporating power of the air upon the soil moisture content in these partially controlled plant cultures may be seen from comparisons of the seasonal marches of these three factors, which are shown as graphs, for Oakland and for Easton, in figures 5 and 6, respec-

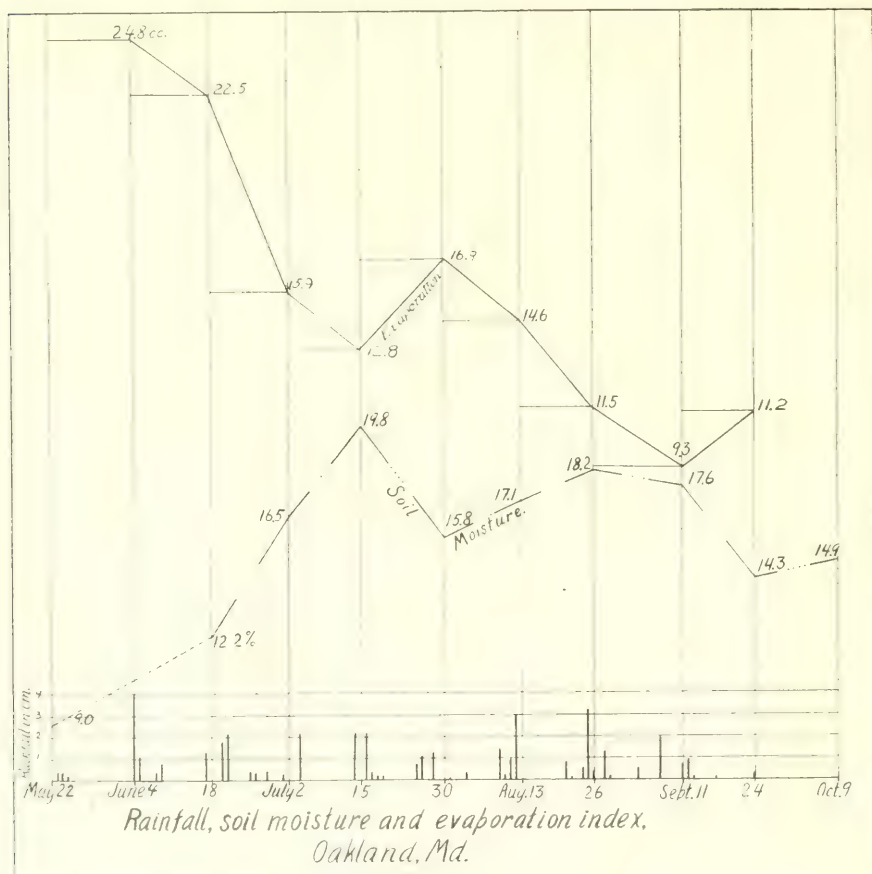


FIG. 5

tively. The abscissas here represent time and season, and the ordinates (the values of the various data) are shown at the dates on which measurements were taken. The daily amounts of rainfall are represented at the bottom of each figure, as vertical lines, the relative lengths of which indicate the depths of rainfall. Soil moisture is given as percentage on the dry weight of the soil as basis. Each ordinate represents the average of all

samples taken on the same day, these values being obtained from tables V and VI. Soil moisture data for June 4 at Oakland (fig. 5) are lacking, since the pots were saturated by rain at the time of observation, and the graph is drawn as a straight, broken line between the point for May 22 and that for June 18. Evaporation is expressed as the average rate of loss from the standard cylindrical porous cup, in cubic centimeters per day, for each culture period of approximately two weeks. The duration of the period to which each rate applies is indicated by the length of the horizontal line drawn to the left of the corresponding point on the graph of

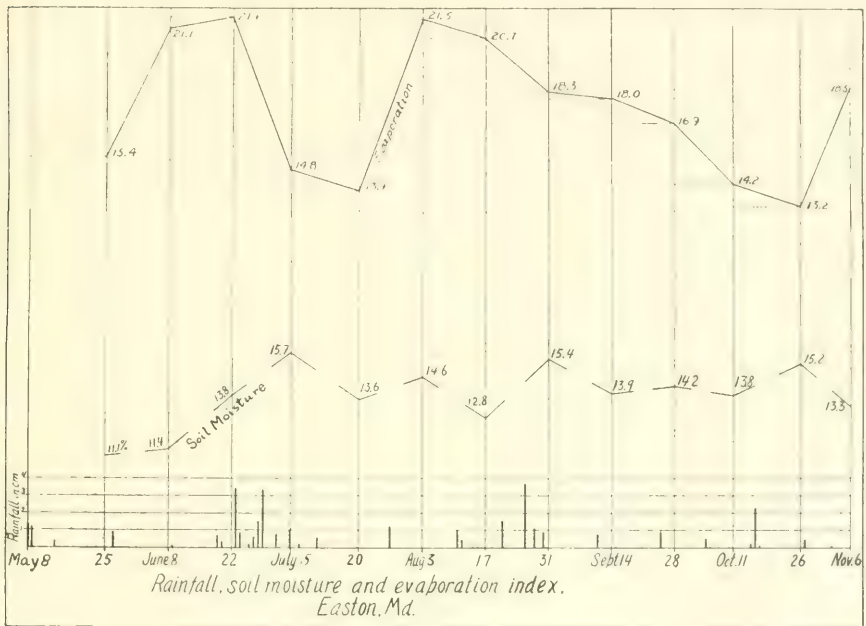


FIG. 6

evaporation. Evaporation was not measured for the last two-week period at Oakland (Oct. 9, Fig. 5), since the atmometer cups had been broken by freezing.

These graphs show an evident relation between rainfall and soil moisture, which is especially apparent in figure 5, for Oakland. Here the highest observed moisture content occurred on July 15, the day after a rain of 2.16 cm. (0.85 inch), and the other high values, for August 26 and September 11, were likewise due to rains, occurring in each case on the same day as that on which the soil sample was taken. These instances indicate that the effects of rain might persist in the culture pots for at least as long as two days. This is not always necessarily the case, however, as is shown by the

observation for June 18. A rain of 1.27 cm. (0.5 in.) occurred on June 17, but the soil moisture content on the next day was somewhat below the average, being only 12.2 per cent. This rapid decrease in soil moisture was probably due to a high rate of loss by evaporation during the period between the cessation of the rain and the time of the observation. The general effect of high evaporation rates in reducing the soil moisture content of the culture pots is suggested in the seasonal marches shown by these graphs for Oakland. In every instance, excepting one (Aug. 26-Sept. 11), the graph for soil moisture slopes in the direction opposite to the direction of slope shown by the graph for evaporation for the corresponding time period. A similar relation is distinguishable in the graphs of figure 6, for Easton, though the relation between the two is here not so clear as in the other case. Soil moisture determinations were not made frequently enough, however, to show the detailed seasonal march of this condition in the cultures, and an exact study cannot be carried out in this connection. The evidence presented indicates clearly that the soil moisture content was greatly influenced by both rainfall and evaporation, in spite of the employment of the irrigators.

The probable effect of a given amount of rain upon the moisture content of the soil in one of these culture pots may be approximately computed from the amount of water required to increase the moisture content from the value of the average maintained by the auto-irrigators to a value representing the maximum water-retaining power of the soil-mass in the pots. The difference between these two latter values may be taken as the amount of water that, falling upon the exposed soil surface of the pot, would cause the maximum increase in soil moisture content. Run-off if not important in this calculation, as the side walls of the pots, extending a centimeter above the soil surface, effectively prevented its occurrence, excepting for the very heaviest rains, and drainage of water through the bottom of the pot should not be very pronounced until the soil mass had become moistened to above its maximum water-retaining power. The average dry weight per cubic centimeter of the soil, as calculated from fifty-two samples each containing about 22.4 cc., proved to be 1.4 g. The 1650 cc. of soil in each pot thus weighed about 2300 g., in the dry state. The maximum water-retaining power of the soil here used, as shown by the samples taken soon after rains, was nearly 23 per cent., on the basis of the dry weight of the soil, while the average moisture content maintained in the pot between rains, by the irrigators, was about 14 per cent. Therefore, 207 cc. of water, added to the soil in its normal condition in the culture, would be all that could be retained against the downward attraction of gravity, for this amount corresponds to an increase in soil moisture content of 9 per cent., on the basis of dry weight. The area of the top of a pot of the type here used is approximately

200 sq. cm., so that a depth of rainfall of 1.03 cm.<sup>15</sup> (0.415 inches) received by a culture, after a period of drought, would be sufficient to bring the soil to its maximum water content, 23 per cent. On the basis of this calculation, the cultures at Oakland must have been saturated by at least ten storms during their combined growth period of twenty weeks, and those at Easton by seven or eight storms during their combined period of twenty-six weeks. Every light rain must also have exerted some influence upon the soil moisture. Under such varying conditions, the fortnightly soil moisture determinations become of little significance, except to show the general magnitude of the variations occurring during the season, and to indicate the relations holding between these and their external causes.

As has been emphasized by Livingston and Hawkins, the environmental moisture conditions influencing plant growth, neglecting the influence of sunshine, may be considered as represented by the relation between the power of the soil to supply water to the plant roots and the power of the aerial environment to remove water by transpiration. Not being able as yet to measure the former of these two terms, but with due regard to the prime importance of the soil moisture content in determining the power of the soil to deliver water to plant roots (especially in such cultures as those here dealt with, where the soil was all alike excepting for its moisture content), an approximation of this value may be obtained by substituting the main component factor for the whole term of water-supplying power. This approximation may then be stated: the entire moisture relation of these plants is approximately represented by the relation between soil moisture content and the evaporating power of the air. This relation (expressed as a ratio) has been employed with considerable success by Shreve,<sup>16</sup> in studies of the relation between climatic and soil conditions, on the one hand, and plant distribution on the other. But such a ratio cannot be used in the present studies, since, as has been remarked, the soil moisture contents of the pots were not determined frequently enough to supply the needed data. From the information above set forth, and on general *a priori* grounds, a still less precise approximation may be attained by substituting in the place of the soil moisture content the main factor tending to increase this content, namely rainfall. Thus modified, the above statement becomes: the moisture relation of these plants may be approximately expressed as the ratio of rainfall to the evaporating power of the air. This ratio, as will be seen at once, involves nothing but climatic conditions, and both of these two conditions were measured in the present study. Comparisons between such ratios for different time periods and for different localities should show, in a general way, the relative tendencies of the different climatic complexes to maintain

<sup>15</sup> This estimate is too high, since the retaining power of the soil was determined with a 1-cm. soil column and the column in the pot was much higher than this.—B. E. L., *Ed.*

<sup>16</sup> Shreve, F., Rainfall as a determinant of soil moisture. *Plant World* 17: 9-26. 1914.

water in any water-absorbing substance (such as soil or a plant). The ratio here proposed is the reciprocal of the ratio of evaporation to rainfall, as used by Transeau<sup>17</sup> which expresses the drying tendency of the climatic environment. It is of course not important which form of ratio is employed, since one is the reciprocal of the other, but the writer finds it easier to think of an influence tending to moisten or to maintain moisture in an object than to think of an influence tending to withdraw water.

In using rainfall as a measure of the general supply of moisture for plants, it is assumed that all of the water falling is effective to increase the moisture of the soil, which is the direct source from which plants absorb water. This assumption is not ordinarily strictly true, however, as has been clearly pointed out by Shreve, and as has been demonstrated also for the pot cultures here employed. As shown above, any rain in excess of 1.03 cm., occurring in a single shower, should have been without effect upon the soil moisture content in these cultures, this moisture content being already at its maximum. Thus, the rainfall-evaporation ratios as here derived are probably generally too high.

#### TEMPERATURE CONDITIONS AND THEIR MEASUREMENT

*Maximum and minimum temperature readings.* The temperature data used in this study were all obtained from maximum and minimum thermometers read daily at sunset. The results of these readings are shown graphically in figure 7, in which the abscissas represent the successive dates of the daily observations, and the ordinates are the recorded maximum and minimum temperatures, in degrees Fahrenheit. The upper two graphs of figure 7 show the maximum and minimum temperatures recorded at Oakland during the period of observation, and the lower two present the corresponding data for Easton.

The temperature conditions at the two stations, as shown in figure 7, exhibited large and irregular fluctuations, this being especially true of the western station. Both the daily range of temperature (within each 24 hour period) and the interdiurnal fluctuations (variations of the average conditions for different 24 hour periods) were greater at Oakland than at Easton.

#### TEMPERATURE WEIGHTINGS AND INTEGRATIONS

*General discussion.* It is obvious that this complex mass of temperature data must be greatly simplified before it may be compared to the plant growth measurements, which were obtained only at relatively long intervals. Two general lines of procedure have been proposed for simplifying such complex series of temperature observations, (1) the grouping of the

---

<sup>17</sup> Transeau, E. N., Forest centers of eastern North America. Amer. Nat. 39: 875-889. 1905.

temperature data into several classes and the computation of the lengths of the time periods during which each class obtains, and (2) the summation of the temperature data for certain seasonal periods. The method of temperature classes was developed by Koeppen<sup>18</sup> and has been modified in various ways by later writers.<sup>19</sup>

In making such temperature summations as those just mentioned either the data for any given time period may be employed directly or they may be replaced, before the summation is performed, by measures of the temperature efficiency, obtained by weighting each temperature magnitude in accordance with its observed or probable effectiveness in promoting plant

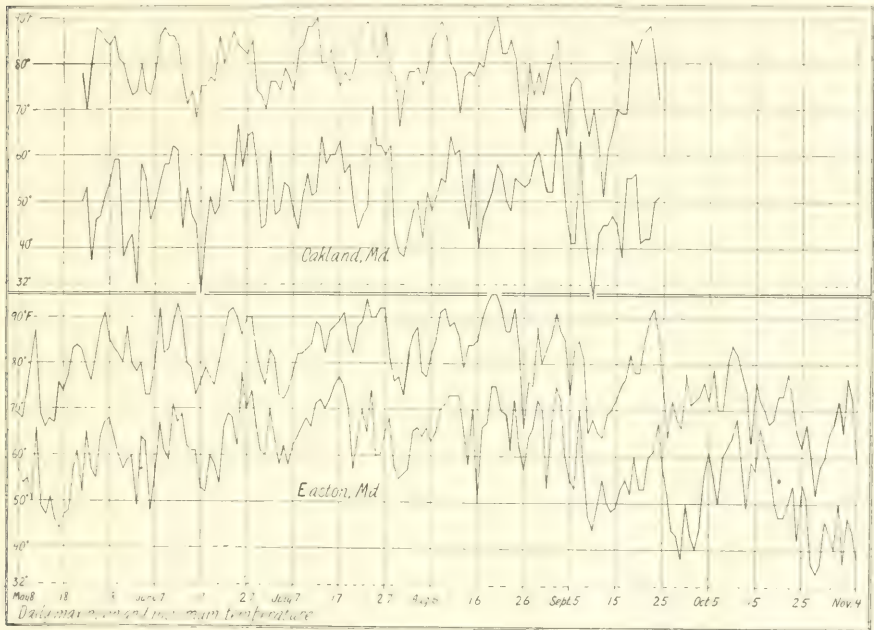


FIG. 7

growth. Until more real information is at hand regarding the effects of different degrees of temperature upon growth processes, such methods are mainly of value in defining and comparing climates as such, for which purpose they have been employed by both of the writers mentioned above.

Another somewhat similar method of simplifying temperature data is that proposed by MacDougal.<sup>20</sup> The average hourly rates of growth are

<sup>18</sup> Koeppen, W., *Die Wärmezonen der Erde, nach der Dauer der heissen, gemässigten und kalten Zeit und nach der Wirkung der Wärme auf die organische Welt betrachtet.* Meteorol. Zeitschr. 1: 215-226. 1884.

<sup>19</sup> See especially: Zon, R., *Meteorological observations in connection with botanical geography, agriculture and forestry.* Monthly Weather Rev. 42: 217-223. 1914.

<sup>20</sup> MacDougal, D. T., *The auxo-thermal integration of climatic complexes.* Amer. Jour. Bot. 1: 186-193. 1914.

to be computed for some given plant form, for time periods during which the temperature ranges over specific intervals on the thermometer scale (intervals such as 40 to 45°, 45 to 50° F., etc.). Then the number of hours representing the duration of natural temperatures lying within each of the given ranges, for any given longer period (day, week, month, growing season, etc.) is ascertained from thermograph records, and the product of the average hourly growth rate for each range by its number of hours is considered as the total temperature efficiency for that range of temperature conditions. Such efficiencies are computed for all of the temperature ranges experienced during the time period considered, and the sum of all of these efficiencies represents the total temperature efficiency of the larger period, for promoting plant growth. The early literature referring to temperature integrations is reviewed by Abbe,<sup>21</sup> and need not be considered further here.

Laboratory studies carried out with plants have shown that plant growth rates are more or less directly related to temperature, the best study bearing on this matter being the recent one of Lehenbauer.<sup>22</sup> If plants are subjected to temperatures of different magnitudes maintained for definite time periods, all other conditions being kept approximately uniform, the growth rates are found to vary in a characteristic manner with the temperature conditions. Below a certain low temperature and above a certain high temperature no growth takes place, these limits being termed the minimum and maximum temperatures for growth. If the growth rates for any plant form, when subjected to different temperatures, are expressed in the form of a graph, the temperature values being the abscissas and the growth rates of the plants being the ordinates, then the graph takes the form of a curve which shows a slow increase in rate of growth for temperatures just above the minimum, followed by a rapid increase until the highest growth rate is approached. Then the increase in growth rate with rise in temperature becomes slow again until the highest point of the graph is reached (the latter point being termed the optimum), when the growth rate rapidly descends to zero with still further increase in temperature. Whether each of the temperatures experienced during the constantly varying conditions ordinarily encountered out-of-doors will affect the growth of plants in the same manner as does a similar maintained temperature, as usually employed in laboratory tests, may only be found out by actual trial. It may be tentatively supposed, however, that plants respond to the varying temperatures of nature in a manner at least similar to that in which they react to maintained temperatures, and indices formulated on this basis

<sup>21</sup> Abbe, Cleveland, First report on the relation between climate and crops. U. S. Weather Bureau, Bull. 36. 1905. Pages 169-343.

<sup>22</sup> Lehenbauer, P. A., Growth of maize seedlings in relation to temperature. *Physiol. Res.* 1: 247-288. 1914. Earlier references to the general subject are there given.

may be tested by comparing them to actual rates of plant growth, as observed out-of-doors.

The use of direct summations of the daily, etc., temperature values is based on the supposition that, within the limits of the temperature range encountered in any given set of investigations, the rates of the plant process studied are approximately proportional to the indices of temperature efficiency obtained by such summations. In other words, if a graph is constructed to show the relation holding between temperature index values and growth rates, the indices of temperature being abscissas and the growth increments for corresponding periods being ordinates, then the graph should take the form of a straight line. Laboratory studies of the elongation of roots and shoots of seedlings indicate that the relation between growth rate and temperature is not really a linear one, but that the linear relation is approached excepting near the optimum and minimum. Thus, for what may be regarded as medium temperatures (intermediate between minimum and optimum) for a given plant form, temperature indices derived from summations may be expected to approximate the actual efficiencies of the corresponding temperatures.

Many workers have sought a general law which would express the relation of physiological processes in general (including plant growth) to temperature conditions. The chemical principle of van't Hoff and Arrhenius (which states that the velocity of many chemical reactions somewhat more than doubles for each rise of  $18^{\circ}$  F. in the temperature of the medium) has been found to apply quite well to several physiological processes, such as photosynthesis, respiration, germination of seeds, etc., but, of course, only within certain limits of temperature. The literature bearing upon the application of this principle has been reviewed by Livingston and Livingston,<sup>23</sup> who propose the use of temperature indices computed on the basis of the van't Hoff-Arrhenius law, employing a temperature coefficient of 2, and considering the rate of growth at  $40^{\circ}$  F. as the unit of temperature efficiency. As pointed out by these writers, such indices may not be expected to hold for all temperatures or for all plants.

Temperature efficiencies based upon the application of the van't Hoff-Arrhenius principle take account of the fact that the relation thus supposed to hold between temperature and plant growth is not at all a linear one. Such efficiencies, expressed graphically, present a logarithmic curve. According to the van't Hoff-Arrhenius principle an increase of one degree in temperature is more effective with relatively high temperatures, in accelerating chemical reactions, than is a similar increase with lower temperatures. As we have seen, laboratory studies of plant growth show that the increase in rate of growth is also accelerated with increase in temperature, up to a

---

<sup>23</sup> Livingston, B. E., and G. J. Livingston, Temperature coefficients in plant geography and climatology. *Bot. Gaz.* 56: 346-375. 1913.

certain limit. Out-door plants, subjected to varying conditions, may be expected to respond to temperature variations in a manner which accords more nearly with the van't Hoff-Arrhenius principle, than with the principle which supposes that the growth rates are proportional to the temperatures themselves, above a certain point on the thermometer scale.

*Direct summations of daily means above 40° F.* Direct summations of temperature readings appear to have considerable value, as a means for roughly comparing the temperature conditions affecting plants growing out-of-doors, and have therefore been computed for the various growth periods of the plant cultures used in the present investigation. To reduce the temperature readings on the Fahrenheit scale to effective temperatures for the growth of soy-bean, a daily mean temperature value of 40° F. was here taken as the minimum or zero for growth. No experimental data appear to be on record in the literature, upon the relation of the growth rate of soy-bean to temperature, but it has been found for many other plants that growth ceases, with falling temperature, at approximately 40° to 43° F., and therefore 40° may be taken as the minimum for the plants here used, without introducing the probability of great error. Having thus established a minimum, the effective temperature values were computed by subtracting 40 from each successive daily mean. The remainders thus obtained for the successive days of each period were added, and the sum of the effective day-degrees thus obtained was treated as a measure of the temperature efficiency for the period. Such direct summations were made for all of the culture periods and for both stations here dealt with, and the results are given in tables IX-XII, which will be described below.

*Temperature summations obtained by use of the chemical coefficient.* The chemical temperature efficiencies proposed by Livingston and Livingston may be computed from the data of ordinary fluctuating outdoor temperatures in at least three ways: (1) The efficiency index of the average temperature for the whole period may be multiplied by the number of days in the period and the product thus obtained may be treated as the total efficiency. If the van't Hoff-Arrhenius law is applicable to the growth of plants subjected to the fluctuations of temperature encountered in the investigation here reported, then the temperature efficiency for each culture period, computed in the manner just described, should exhibit at least as close a correlation to the corresponding growth rate as does the corresponding direct summation of effective temperatures. (2) The indices for the successive daily mean temperatures may be summed for each period, thus giving due weight to the interdiurnal variations in temperature. (3) The indices for the maximum and minimum temperatures may be averaged for each day and the averages thus obtained may be summed throughout the period. In this way account is taken, not only of the interdiurnal variations but also of the daily range of temperature. Each of these three methods of computa-

tion has been employed for each of the culture periods and for each of the stations here considered, the results being summarized in tables IX-XII, which will be considered below.

#### LIGHT CONDITIONS AND THEIR APPROXIMATION

The present state of our knowledge regarding the relations to plant growth, of the different wave-lengths of radiant energy that we term light, is still less satisfactory than is our knowledge regarding the temperature relation of plants. The portions of the solar spectrum that are most effective in promoting certain plant processes are recognized in a general way, but the quantitative aspects of the problem here brought up have been but little investigated. Most of the studies that have been carried out in this connection have dealt with sunlight, and have treated the solar radiation as if it were constant in composition, if not in intensity, but of course it is not constant in either respect. Furthermore, while it is well known that only the light of certain portions of the solar spectrum is important for plant growth, the only data available for the evaluation of the sunlight for any locality for any period of time are rather rough, general estimates of the duration and of the heating effect of the total radiation received.

The duration of sunshine in hours per day (that is, the duration of light with a heating effect above a certain very roughly defined minimum) is measured by the U. S. Weather Bureau only at its regular stations. The sunshine records for the cooperative stations, such as Oakland and Easton, are only the local observer's ocular estimates of clear, partly cloudy and cloudy days. This latter form of record does not appear to be sufficiently precise, nor is it sufficiently detailed, to furnish data for a study of the relations between plant growth and light conditions. Fortunately, however, complete instrumental records of sunshine duration are available for other stations in the vicinity of those here used. Thus the data for Elkins, West Virginia, have been here employed as if for Oakland, and the data for Washington, D. C., and Baltimore, Maryland, have been averaged, the average being used as if for Easton. The general character of the weather at Oakland is very similar to that at Elkins, the two stations being situated at similar altitudes and about 48 km. (30 mi.) apart. Likewise, the weather conditions at Easton are generally not markedly unlike those at Baltimore and Washington, which are only about 50 and 75 km. (30 and 45 mi.) distant. The averages obtained by combining the records for Baltimore and for Washington were compared to the local observer's records at Easton day by day, and the two sets of data showed very few disagreements as to the character of the days, whether clear or cloudy.

All the sunshine data are presented in tables VII (Oakland) and VIII (Easton), to which reference will be made in the following paragraphs.

For every culture period there are shown in these tables two approximations of the amount of sunshine available to the plants: (1) the average daily duration of actual sunshine as derived from the average daily duration of possible sunshine for that period and latitude, and from the local observer's records, and (2) the average daily duration of actual sunshine recorded by the sunshine-recorder at Elkins (for Oakland), and the mean of the numbers thus recorded at Baltimore and Washington (for Easton).

In the case of the local records of the cooperative observers, days recorded as clear were treated as whole days of sunshine (column 4), partly cloudy days were treated as half-days of sunshine (column 5), and cloudy days were treated as without any sunshine (column 6). These days and half-days were summed for each culture period for each station, and the sum was expressed (column 8) as percentage of the total number of days in the culture period (column 3). Next, the average daily duration of possible sunshine for this region,<sup>24</sup> for each period (column 7), was multiplied by the corresponding observational percentage (column 8), to give an approximation of the average daily duration of *actual* sunshine at the two stations considered (table VII, column 10; table VIII, column 12).

For each day of the Oakland season the percentage of possible sunshine duration was obtained from the records of the instrument at Elkins. These daily percentages were then averaged for each Oakland culture period, the resulting average daily percentages being given in table VII, column 9. Each average daily possible duration of sunshine (table VII, column 7) was next multiplied by the corresponding average daily percentage (table VII, column 9), to give an approximation of the average daily actual duration of sunshine (table VII, column 11), for each culture period at Oakland.

The percentages of possible sunshine duration for each Easton period were obtained from the instrumental records for Washington and for Baltimore (table VIII, columns 9 and 10), and these two data were then averaged for each period (table VIII, column 11). These composite percentages were each multiplied by the corresponding average daily possible number of hours of sunshine (table VIII, column 7), to give a number that is taken to represent the average daily actual duration of sunshine (table VIII, column 13) for each culture period at Easton. Since the cultures at this station were shaded from possible sunshine for approximately four hours each day, as has been noted, the data of column 13 require to be corrected so as to take this into account. This was done by subtracting from each number in column 13 four times the corresponding percentage in column 11, since only this percentage of the four hours when the plants might have been in shade is to be considered as sunshine period. The numbers thus corrected are given in column 14.

---

<sup>24</sup> Derived from data in: Sunshine tables, U. S. Dept. Agric. Weather Bureau.

It appears that the numbers representing average daily actual sunshine as obtained by the first method (from local observers' records) are generally somewhat larger than those obtained by the second method (from instrumental records for nearby stations). Which of these two series may be more nearly correct cannot be determined, however; both methods are very crude. The data derived by the second method (table VII, column 11; table VIII, column 14) will be employed where sunshine duration enters into the following discussions, but it is possible that the first method may prove of some value in climatic studies related to plant growth, in cases where ocular observations of cloudiness constitute the only available sunshine data.

The sunshine records of the regular U. S. Weather Bureau stations, such as Elkins, Baltimore and Washington, are obtained by means of Marvin sunshine recorders, each of which consists of a pair of thermometers, so arranged as to record the lengths of the periods during which the heating power of the sunlight received is sufficiently great to maintain or surpass a certain difference in temperature between a blackened and a transparent glass bulb. The instrument thus records sunshine when the sun is shining brightly, but does not take account of differences in the absolute intensities of solar radiation received, except to show that the heating power is above the threshold value for the instrument. On account of the form of its inclined cylindrical bulbs, this threshold value does not occur with the same light intensity for different hours of the day nor for different seasons of the year. This kind of sunshine record is, however, the best that is obtainable at the present time, and it may be supposed to have some possible value as indicator of favorable or unfavorable conditions for plant growth.

The studies of Richter<sup>25</sup> indicate that photosynthesis in plants, and consequently the rate of formation of carbohydrates (which generally constitute the greater part of the dry substance of these organisms) is proportional to the amount of light energy actually absorbed by the leaves. From this it may be supposed that the sunshine data here considered may indicate, in a general way at least, the relative amounts of radiant energy available for plants during the different time periods, providing the quality of the sunlight does not vary greatly.

Studies on the effect of shading upon plant growth, by Lubimenko<sup>26</sup> Combes,<sup>27</sup> Rose,<sup>28</sup> and others indicate that moderate variations in light intensity may be accompanied by very great differences in plant growth. In most

---

<sup>25</sup> Richter, A., Etude sur la photosynthèse et sur l'absorption par la feuille verte. *Rev. gen. Bot.* **14**: 151-169, 211-218. 1902.

<sup>26</sup> Lubimenko, W., Production de la substance seche et de la chlorophyll chez les vegetaux superieures aux differents intensités lumineuses. *Ann. Sci. Nat. Bot.* **IX**, 7: 321-415. 1908.

<sup>27</sup> Combes, R., La détermination des intensités lumineuses optima par les vegetaux au divers stades de developpement. *Ann. Sci. Nat. Bot.* **IX**, 11: 74-254. 1910.

<sup>28</sup> Rose, Edmond, L'énergie assimilatrice chez les plantes. *Ann. Sci. Nat. Bot.* **IX**, 17: 1-110. 1913.

TABLE VII  
*Computations of duration of sunshine for the Oakland station*

NO. OF CLIMATIC PERIOD	BEGINNING AND END OF PERIOD	LENGTH OF PERIOD	NO. OF CLOUDY, PARTLY CLOUDY, AND CLEAR DAYS IN PERIOD <sup>a</sup>			AVERAGE POSSI- BLE SUNSHINE DURATION PER DAY FOR LATI- TUDE OF OAKLAND	PERCENTAGE OF POSSIBLE SUNSHINE DURATION		AVERAGE ACTUAL DURATION OF SUNSHINE PER DAY		AVERAGE MAXI- MUM RADIATION RECEIVED PER DAY AT MT. WEATHER (ALTITUDE 1750 FT.) <sup>c</sup>
			Clear	Partly Cloudy	Cloudy		For Oakland <sup>a</sup>	For Elkins, W. Va. b	For Oakland <sup>a</sup>	For Elkins, W. Va. b	
		days	days	days	hours	per cent.	per cent.	hours	hours	Gram-calories	
1	May 23-June 4	13				14.5	71	71	10.3	765	
2	June 5-18	14	6	8	0	14.7	71	73	10.4	770	
3	June 19-July 2	14	6	6	2	14.7	64	68	9.4	752	
4	July 3-15	13	6	4	3	14.6	62	57	9.1	730	
5	July 16-30	15	9	4	2	14.3	73	63	10.4	705	
6	July 31-August 13	14	8	3	3	13.9	68	52	9.5	683	
7	August 14-26	13	9	2	2	13.3	77	58	10.2	630	
8	August 27-September 11	16	7	6	3	12.7	63	41	8.0	580	
9	September 12-24	13	8	3	2	12.2	68	69	8.9	520	
10	September 25-October 9	14				11.5		67		470	
Average									9.5	8.5	

<sup>a</sup> Data based on ocular observations of clear, partly cloudy and cloudy days at Oakland.

<sup>b</sup> Data based on instrumental records at Elkins.

<sup>c</sup> Data based on Kimball's graph.

TABLE VIII  
*Computations of duration of sunshine for the Easton station*

NO. OF CULTURE PERIOD	BEGINNING AND END OF PERIOD	LENGTH OF PERIOD	NO. OF CLEAR, PARTLY CLOUDY, AND CLOUDY DAYS IN PERIOD <sup>a</sup>			AVERAGE POSSIBLE SUNSHINE DURATION PER DAY FOR LATITUDE OF EASTON		PERCENTAGE OF POSSIBLE SUNSHINE DURATION				AVERAGE ACTUAL DURATION OF SUNSHINE PER DAY		AVERAGE MAXIMUM RADIATION RECEIVED PER DAY AT MT. WEATHER (ALTITUDE 1750 FT.). <sup>f</sup>	
			Clear	Partly Cloudy	Cloudy	hours	per cent.	For Easton <sup>a</sup>	For Washington, D. C. b	For Baltimore, Md. c	Average for Washington and Baltimore combined, c	For Easton <sup>a</sup> and Baltimore combined, c			
												hours	per cent.		
1	May 9-25.....	17	13	3	1	14.2	85	84	74	79	12.1	11.2	750		
2	May 26-June 8.....	14	14	0	0	14.6	100	72	66	69	14.6	10.1	765		
3	June 9-22.....	14	11	1	2	14.7	82	62	54	58	12.1	8.5	770		
4	June 23-July 6.....	14	8	4	2	14.7	71	56	52	54	10.4	7.9	745		
5	July 7-20.....	14	8	5	1	14.5	75	66	60	63	10.9	9.1	725		
6	July 21-August 3.....	14	6	7	1	14.3	68	77	67	72	9.7	10.3	690		
7	August 4-17.....	14	10	2	2	13.6	78	73	60	66.5	10.6	9.0	675		
8	August 18-31.....	14	10	0	4	13.0	71	63	58	60.5	9.2	7.9	610		
9	September 1-14.....	14	9	3	2	12.7	75	68	68	68 <sup>d</sup>	9.5	8.6	570		
10	September 15-28.....	14	10	3	1	12.1	82	76	76	76 <sup>d</sup>	9.9	9.2	505		
11	September 29-October 11.....	13	6	4	3	11.5	62	63	59	61	7.1	7.0	455		
12	October 12-26.....	15	7	3	5	11.0	57	49	51	50	6.3	5.5	400		
13	October 27-November 6.....	11	9	1	1	10.3	86	88	63	70.5	8.8	7.2	380		
Average.....													10.1	8.6	5.9

<sup>a</sup> Data based on on-ocular observations of clear, partly cloudy and cloudy days at Easton.

<sup>b</sup> Data based on instrumental records at Washington.

<sup>c</sup> Data based on instrumental records at Baltimore.

<sup>d</sup> The number for Baltimore is used, in the absence of that for Washington.

<sup>e</sup> The cultures at Easton were shaded by trees about four hours per day, which is here taken into account.

<sup>f</sup> Data based on Kimball's graph.

of these experiments, however, the conditions affecting the evaporating power of the air and the transpiration rates were not precisely enough measured or defined to make it certain that the differences in growth attributed to differences in light were indeed entirely light affects.

In addition to the sunshine records, the seasonal march of solar radiation in general is shown in the final columns of tables VII and VIII. These data are from Kimball's<sup>29</sup> graph of the maximum daily amount of radiation received upon a horizontal surface during each decade of the year, and is based on three years' record at Mt. Weather, Va. The latter station has an altitude of about 520 m. (1750 ft.) above sea-level, and is therefore fairly comparable to Oakland, Md. The main value of this Mt. Weather record for the present purpose, however, is to show the seasonal march of the average maximum intensity per day, of solar radiation, for each culture period, for this general region. Thus, the average daily maximum radiation for culture period 3 for Easton appears to be more than twice as great as that for period 13 for the same station, and a similar relation holds, of course, for Oakland. These differences in the extreme values of total solar radiation per day are partly determined by the actual differences in radiation intensity at different seasons of the year, and partly by corresponding differences in the lengths of the daily periods of daylight.

#### PRESENTATION OF WEATHER DATA

All of the weather data here considered have been computed as summations or averages for periods corresponding to the first two weeks (approximately) of each full culture period, and also for each entire culture period of about four weeks, and the resulting values are given in tables IX-XII, which have already been mentioned. Tables IX and X refer to Oakland, tables XI and XII to Easton. Tables IX and XI show the data for the first two weeks (approximately) of each full culture period. The first line gives the serial numbers of the cultures, as heretofore used. Lines 2 and 3 give the dates of the beginning and end of each two-week period. Line 6 gives the direct summation of the daily mean temperature values in terms of degrees Fahrenheit above 40°F., for each period. The average daily mean effective temperature (line 7) is derived by dividing each number in line 6 by the number of days in the period (line 4). Line 8 shows the average daily range of temperature for each two-week period, this being the difference between the average daily minimum and the average daily maximum.

---

<sup>29</sup> Kimball, Herbert H., The total radiation received on a horizontal surface from the sun and sky at Mount Weather. *Monthly Weather Rev.* 42: 474-487. 1914. (See especially fig. 8, page 484.)

Lines 9 to 12 give temperature efficiency indices for each period, computed in three different ways, according to the general method proposed by Livingston and Livingston, employing the chemical temperature coefficient. (1) To obtain the values given in line 9, the average daily mean temperature for the two-week period in question was first found, and then the efficiency index corresponding to this value was obtained directly from the table given by Livingston and Livingston<sup>30</sup> (page 366). To give the efficiency indices for the entire period (line 10), each number given in line 9 was multiplied by the corresponding number of days (line 4). (2) To obtain the temperature efficiency values given in line 11, the mean temperature for each day of the period in question was found by averaging its maximum and minimum. The efficiency index corresponding to each of these daily means was then found from the Livingston and Livingston table, and these indices were finally summed. (3) The values given in line 12 were derived in still another way. The efficiency index corresponding to the maximum and that corresponding to the minimum were found, from the table just mentioned, for each day of the period considered, and these two indices were averaged. The average daily efficiency indices thus obtained, were finally summed.

Lines 13 and 15 exhibit the total amount of rainfall for each two-week period, in centimeters and in inches. Line 14 gives the average daily rainfall for the period, in centimeters. Line 16 shows the total evaporation for the period, in cubic centimeters, from the cylindrical porous cup atmometer, the readings having been first corrected to the Livingston cylindrical standard, while line 17 gives the average daily evaporation for the period. Line 18 gives the value of the rainfall-evaporation ratio for each period, this being obtained by dividing each number in line 13 by the corresponding one in line 16. Line 19 indicates the total number of hours of sunshine recorded by the Marvin sunshine recorder at Elkins, W. Va. (representing Oakland) or the average of the similar records obtained at Baltimore and at Washington (representing Easton), for each period. Each value in line 19 divided by the number of days in the period (line 4) gives the corresponding value in line 20, which is the average daily duration of sunshine for the period.

Tables X and XII have the same form as tables IX and XI, and all of the description just given applies also to these, excepting that the data of tables X and XII refer to the full culture periods of approximately 4 weeks instead of to the first two-week portion of each full period.

---

<sup>30</sup> These efficiency indices were derived by the authors just mentioned, by considering the growth rate as unity at 40°F. and supposing that this rate doubles with each rise of 18° above 40°F.

TABLE IX  
*Climatic data for the first half (about 2 weeks) of each culture period at Oakland*

	1 May 23 June 4 13	2 June 5 June 18 14	3 June 19 July 2 14	4 July 3 July 15 13	5 July 16 July 30 15	6 July 31 Aug. 13 14	7 Aug. 14 Aug. 26 13	8 Aug. 27 Sept. 11 16	9 Sept. 12 Sept. 24 13	Average ..... ..... 14
(1) Culture number.....										
(2) Beginning of period.....	64.7	64.3	67.6	67.0	67.7	65.4	65.5	62.1	60.7	65.0
(3) End of period.....	321	340	387	351	416	355	331	354	269	347
(4) Length of period, days.....	24.7	24.3	27.6	27.0	27.7	25.4	25.5	22.1	20.7	25.0
(5) Average daily mean temperature, <i>degrees F.</i> .....	30.1	27.8	24.4	28.5	25.3	28.4	29.0	23.3	27.2	27.1
(6) Sum of effective daily mean temperatures, above 40°F.....	2.62	2.52	2.94	2.83	2.94	2.62	2.62	2.33	2.25	2.62
(7) Average daily mean effective temperature above 40°F.....										
(8) Average daily range of temperature, <i>degrees F.</i> .....										
(9) Efficiency index corresponding to average daily mean.....										
(10) Efficiency index corresponding to average daily mean × no. of days.....	34.1	35.3	41.2	36.8	44.0	36.7	34.0	37.3	29.2	36.5
(11) Sum of daily efficiency indices.....	33.2	37.1	41.8	37.4	45.2	37.9	36.6	39.4	29.9	37.6
(12) Sum of mean daily efficiency indices.....	39.7	40.9	46.3	43.1	50.1	43.4	40.9	42.4	34.7	42.4

(13) Total rainfall ( $R$ ), <i>cm.</i> .....	5.00	2.28	6.65	6.55	3.66	5.76	4.88	4.62	1.30	4.52
(14) Average daily rainfall, <i>cm.</i> ...	0.39	0.16	0.48	0.50	0.24	0.41	0.38	0.29	0.10	0.33
(15) Total rainfall ( $R$ ), <i>inches</i> ....	1.97	0.90	2.62	2.58	1.44	2.27	1.92	1.82	0.51	1.78
(16) Index of evaporation ( $E$ ), <i>cc.</i> .....	323	315	223	166	254	204	150	149	145	2.14
(17) Average daily evaporation, <i>cc.</i> .....	24.8	22.5	15.9	12.8	16.9	14.6	11.5	9.3	11.2	15.5
(18) Rainfall-evaporation ratio $\left(\frac{R}{E}\right)$ .....	0.016	0.007	0.030	0.040	0.014	0.028	0.033	0.031	0.009	0.023
(19) Total duration of sunshine at Elkins, W. Va., <i>hours</i> ....	134	150	140	108	135	101	100	83	109	118
(20) Average daily duration of sunshine ( $S$ ), <i>hours</i> .....	10.3	10.7	10.0	8.3	9.0	7.2	7.7	5.2	8.4	8.5

TABLE X  
*Climatic data for each full culture period (about 4 weeks) at Oakland*

	1 May 23 June 18 27	2 June 5 July 2 28	3 June 19 July 15 27	4 July 3 July 30 28	5 July 16 Aug. 13 29	6 July 31 Aug. 26 27	7 Aug. 14 Sept. 11 29	8 Aug. 27 Sept. 24 29	Average ..... ..... 28
(1) Culture number.....									
(2) Beginning of period.....									
(3) End of period.....									
(4) Length of period, <i>days</i> .....									
(5) Average daily mean temperature, degrees F.....	64.5	66.0	67.3	67.4	66.6	65.4	63.6	61.5	65.3
(6) Sum of effective daily mean tempera- tures, above 40° F.....	661	727	738	767	771	686	685	623	707
(7) Average daily mean effective tempera- ture, above 40° F.....	24.5	26.0	27.3	27.4	26.6	25.4	23.6	21.5	25.3
(8) Average daily range of temperature, degrees F.....	29.0	26.1	26.5	26.9	26.9	28.7	26.2	25.3	27.0
(9) Efficiency index corresponding to av- erage daily mean.....	2.57	2.73	2.89	2.89	2.78	2.62	2.48	2.29	2.66
(10) Efficiency index corresponding to av- erage daily mean × no. of days.....	69.4	76.5	78.0	80.8	80.7	70.7	71.3	66.5	74.2
(11) Sum of daily efficiency indices corre- sponding to daily means.....	70.3	78.9	79.2	82.6	83.1	74.5	76.0	69.3	76.7
(12) Sum of mean daily efficiency indices.....	80.6	87.2	89.4	93.2	93.5	84.3	83.3	77.1	86.1

(13) Total rainfall ( $R$ ), <i>cms.</i> .....	7.28	8.93	13.20	10.21	9.42	10.64	9.50	5.92	9.39
(14) Average daily rainfall, <i>cms.</i> .....	0.27	0.32	0.49	0.37	0.33	0.39	0.33	0.20	0.34
(15) Total rainfall ( $R$ ), <i>inches</i> .....	2.87	3.52	5.20	4.02	3.71	4.19	3.74	2.33	3.70
(16) Index of evaporation ( $E$ ), <i>cc.</i> .....	638	538	389	420	458	354	299	294	424
(17) Average daily evaporation, <i>cc.</i> .....	23.6	19.2	14.4	15.0	15.8	13.1	10.3	10.1	15.2
(18) Rainfall-evaporation ratio ( $\frac{R}{E}$ ).....	0.011	0.017	0.034	0.024	0.021	0.030	0.032	0.020	0.024
(19) Total duration of sunshine at Elkins, W. Va., <i>hours</i> .....	284	291	248	244	235	203	189	197	236
(20) Average daily duration of sunshine ( $S$ ), <i>hours</i> .....	10.5	10.4	9.2	8.7	8.1	7.5	6.5	6.8	8.5

TABLE XI  
*Climatic data for the first half (about 2 weeks) of each culture period at Easton*

	1	2	3	4	5	6	7	8	9	10	11	12	13	Average
(1) Culture number.....	May 9	May 26	June 9	June 23	July 7	July 21	Aug. 4	Aug. 18	Sept. 1	Sept. 15	Sept. 29	Oct. 12	Oct. 27	
(2) Beginning of period.....	May 25	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Aug. 31	Sept. 14	Sept. 28	Oct. 11	Oct. 26	Nov. 6	
(3) End of period.....	May 17	June 14	June 14	July 14	July 14	Aug. 14	Aug. 14	Aug. 14	Sept. 14	Sept. 14	Oct. 13	Oct. 15	Nov. 11	14
(4) Length of period, <i>days</i> .....	64.7	71.4	71.2	74.2	77.0	74.4	75.7	76.1	67.0	66.2	64.1	62.1	52.8	68.9
(5) Average daily mean temperature, <i>degrees F.</i> .....														
(6) Sum of effective daily mean temperatures, above 40° F. ....	419	439	437	478	518	481	499	505	378	367	313	332	141	408
(7) Average daily mean effective temperature, above 40° F. ....	24.7	31.4	31.2	34.2	37.0	34.4	35.7	36.1	27.0	26.2	24.1	22.1	12.8	28.9
(8) Average daily range of temperature, <i>degrees F.</i> .....	23.3	21.9	20.4	17.0	16.1	22.5	18.7	18.1	19.2	23.8	20.7	16.8	23.0	20.1
(9) Efficiency index corresponding to daily mean.....	2.62	3.30	3.30	3.70	4.16	3.70	4.00	4.00	2.83	2.72	2.08	2.33	1.65	3.11
(10) Efficiency index corresponding to average daily mean × no. of days	44.5	46.2	46.2	51.8	58.2	51.8	56.0	56.0	39.6	38.1	27.0	35.0	18.1	43.7
(11) Sum of daily efficiency indices corresponding to daily means....	45.7	48.2	47.9	53.9	59.3	54.1	56.7	58.3	42.6	39.9	34.1	35.8	18.6	45.8
(12) Sum of mean daily efficiency indices.....	50.0	52.3	51.4	56.8	61.7	59.0	60.0	62.2	45.2	44.2	35.9	37.9	17.7	48.8

(13) Total rainfall ( $R$ ), <i>cm.</i> .....	1.6	2.8	1.2	11.7	0.7	1.2	1.4	7.2	0.8	0.9	0.5	2.5	0.5	2.540
(14) Average daily rainfall, <i>cm.</i> .....	0.091	0.20	0.086	0.840	0.050	0.086	0.10	0.51	0.057	0.061	0.039	0.17	0.046	0.18
(15) Total rainfall ( $R$ ), <i>inches</i> .....	0.63	1.09	0.47	4.59	0.29	0.48	0.56	2.82	0.30	0.36	0.20	0.97	0.18	1.0
(16) Index of evaporation ( $E$ ), <i>cc.</i> .....	262	296	304	208	194	299	290	256	252	236	184	198	204	245
(17) Average daily evaporation, <i>cc.</i> .....	15.1	21.1	21.6	14.8	13.9	21.5	20.7	18.3	18.0	16.9	14.2	13.2	18.5	17.5
(18) Rainfall-evaporation ratio $\left(\frac{R}{E}\right)$ .....	0.006	0.009	0.004	0.056	0.004	0.004	0.005	0.028	0.004	0.004	0.003	0.013	0.002	0.011
(19) Total no. of hours of sunshine for culturalures (based on data of Bal- timore and Washington).....	138	102	87	81	92	104	90	76	83	87	60	53	48	84.7
(20) Average daily duration of sun- shine ( $S$ ), <i>hours</i> .....	8.1	7.3	6.2	5.8	6.6	7.4	6.4	5.4	5.9	6.2	4.6	3.5	4.4	5.9

TABLE XII  
*Climatic data for each full culture period (about 4 weeks) at Easton*

(1) Culture number.....	1	2	3	4	5	6	7	8	9	10	11	12	Aver- age
(2) Beginning of period.....	May 9	May 26	June 9	June 23	July 6	July 21	Aug. 4	Aug. 18	Sept. 1	Sept. 15	Sept. 29	Oct. 12	
(3) End of period.....	June 8	June 22	July 5	July 20	Aug. 3	Aug. 17	Aug. 31	Sept. 14	Sept. 28	Oct. 11	Oct. 26	Nov. 6	
(4) Length of period, <i>days</i> .....	31	28	28	28	28	28	28	28	28	27	28	26	28
(5) Average daily mean temperature, <i>degrees F.</i> .....	67.7	71.3	72.7	75.6	75.7	75.0	75.9	71.5	66.6	65.2	63.0	58.2	69.9
(6) Sum of effective daily mean temperatures, above 40° F. ....	858	876	915	996	999	980	1004	883	745	680	645	473	838
(7) Average daily mean effective temperature, above 40° F. ....	27.7	31.3	32.7	35.6	35.7	35.0	35.9	31.5	26.6	25.2	23.0	18.2	29.9
(8) Average daily range of temperature, <i>degrees F.</i> .....	22.6	21.2	18.7	16.6	19.3	20.6	18.4	18.7	21.5	22.3	18.8	19.9	19.9
(9) Efficiency index corresponding to daily mean.....	2.96	3.30	3.50	3.93	3.93	3.85	4.00	3.92	2.78	2.40	2.21	1.99	3.24
(10) Efficiency index corresponding to average daily mean $\times$ no. of days.....	90.7	92.4	98.0	110.0	110.0	107.8	112.0	95.6	77.7	65.1	62.0	53.1	89.5
(11) Sum of daily efficiency indices corresponding to daily means.....	93.9	96.1	101.8	113.2	113.4	110.8	115.0	100.9	82.5	74.0	69.9	54.4	93.8
(12) Sum of mean daily efficiency indices.....	102.3	103.7	108.2	118.5	120.7	119.0	122.2	107.4	89.4	80.1	73.8	55.6	100.1

(13) Total rainfall ( $R$ ), <i>cm.</i> .....	4.4	4.0	12.9	12.4	1.9	2.6	8.6	8.0	1.7	1.4	3.0	3.0	5.3
(14) Average daily rainfall, <i>cm.</i> .....	0.14	0.14	0.46	0.44	0.068	0.095	0.31	0.29	0.061	0.052	0.11	0.12	0.190
(15) Total rainfall ( $R$ ), <i>inches</i> .....	1.72	1.56	5.06	4.88	0.77	1.04	3.38	3.12	0.66	0.56	1.17	1.15	2.09
(16) Index of evaporation ( $E$ ), <i>cc.</i> .....	558	600	512	402	493	589	546	508	488	420	382	402	492
(17) Average daily evaporation, <i>cc.</i> .....	18.0	21.4	18.3	14.4	17.6	21.0	19.5	18.1	17.4	15.6	13.6	15.5	17.5
(18) Rainfall-evaporation ratio ( $\frac{R}{E}$ ).....	0.008	0.007	0.025	0.031	0.004	0.004	0.016	0.016	0.003	0.003	0.008	0.007	0.011
(19) Total no. of hours of sunshine for cul- tures (based on data of Baltimore and Washington).....	239	190	168	174	196	193	165	160	171	146	115	104	168
(20) Average daily duration of sunshine ( $S$ ), <i>hours</i> .....	7.7	6.8	6.0	6.2	7.0	6.9	5.9	5.7	6.1	5.4	4.1	4.0	6.0

## DISCUSSION

The Oakland and Easton stations, as has been stated, were selected for comparative study in this paper because they may be taken to represent the extremes of the climatic conditions encountered in the Maryland area. In the discussion that follows, the various climatic features measured for these two stations during the summer of 1914, will be considered as environmental conditions presumably affecting the growth of the corresponding culture plants, and these paragraphs will present comparisons of plant features with climatic conditions for Oakland and for Easton, as well as comparisons of the Oakland data with those of the other station. An attempt will be made to bring out some of the relations that obtained for the different observation periods at each station, between the soy bean plants and the concomitant and supposedly controlling conditions of their surroundings.

## THE FROSTLESS SEASON

It has already been noted that the season of active plant growth proved to be much shorter at Oakland than at Easton. The frostless season at Oakland, for 1914, began after a severe frost on June 17, and ended with another severe frost on September 28. Thus the duration of this season was 103 days for Oakland. The experiments with which this paper deals were not begun at Easton until May 8, so that the last severe spring frost was not actually encountered at that station, but the records of the local observer show that this probably occurred April 11. The first killing frost in autumn occurred at Easton on November 7, so that the length of the frostless season of 1914 was approximately 210 days for that station. At Oakland the frosts mentioned were severe enough to kill soy bean seedlings in the general region, and the autumn frost actually did kill the plants in the experimental cultures. The same statement holds regarding the season at Easton, except that, since the last spring frost was not actually experienced by the cultures, its severity is merely inferred. The difference between the lengths of the frostless seasons for Easton and Oakland, as here indicated (107 days), appears to be greater than is usual; as is clear from Fassig's<sup>31</sup> data, the normal length of the frostless season for Easton is 201 days and that for Oakland is 134 days, the normal difference being thus only 67 days.

---

<sup>31</sup> Fassig, Oliver L., The period of safe plant growth in Maryland and Delaware. *Monthly Weather Rev.* 42: 152-158, 1914.

COMPARISONS BETWEEN THE PLANT GROWTH VALUES AND CLIMATIC  
INDICES FOR OAKLAND WITH THOSE FOR EASTON, FOR THE  
ENTIRE PERIOD OF OBSERVATIONS

Besides the length of the growing season, many other climatic characters are of course influential in determining plant growth, and some of these will now be considered, in relation to the corresponding growth characters exhibited by the culture plants here dealt with.

The comparative efficiencies of the climatic conditions, as a whole, expressed in terms of the growth of the soy-bean plants here employed, for the two stations and for the entire period of experimentation, are presented in the last column in tables I-IV. All of these are expressed both as average rates per day and as rates per period, excepting those for length and width of mature leaves ( $l$  and  $w$ ), which are given only as rates per period. These measurements of *mature* leaves were obtained in order to determine the influence of the surroundings on the size attained by the leaves when mature, and these data were therefore not related to the rate of leaf expansion; they refer simply to the limit to this expansion set by the surroundings. Therefore average daily rates were not obtained in these two cases. On the other hand, those measurements of leaf length and width that were made for *all* leaves (whether mature or not) and that furnish the data for the leaf-products ( $P$ ), require expression as mean daily rates. These leaf-products proved to be approximate measurements of total leaf surface per plant, developed during their respective periods. The data for two-week periods do not, of course, include actual measurements of leaf area, but in the case of the four-week periods the derived leaf-products and the actually measured leaf areas are both available for comparison (tables II and IV, lines 12 and 14). It is seen at once that these two series of values vary in the same direction across the tables. In order to investigate this parallelism each leaf-product in line 12 was divided by the corresponding leaf area in line 14, thus giving the ratio  $\frac{P}{A}$  (line 18).

If the leaf-products were always actually proportional to the corresponding leaf area, this ratio would have a constant value, and the actual values are seen, indeed, to be nearly constant. The average value of  $\frac{P}{A}$  in table II, for Oakland, is 1.34 and the greatest plus and minus variations from this value are 7.4 and 5.2 per cent., respectively. Similarly this ratio average in table IV, for Easton, is 1.32, with maximum plus and minus variations of 7.5 and 6.1 per cent., respectively. It is therefore very clear that the leaf-product values (derived by measurements that do not involve any injury to the leaves) are to be considered as almost truly proportional to the leaf areas (which cannot be readily obtained without the removal of

the leaves from the plants). Since this conclusion holds so well in the cases where both leaf-products and leaf areas are available, it is probably safe to assume a similar relation in the other cases. Therefore, the leaf-products are here employed as approximate measures of leaf area both for the two-week and for the four-week periods.

The average daily rates of plant growth for the whole season of these studies (measured by the various criteria) are given for both stations in columns 3 and 4 of table XIII, and these columns also include the data of length and width of mature leaves, all data being taken without change from the last column of tables I-IV. Column 5 of table XIII presents the ratios obtained by dividing each value for Oakland by the corresponding value for Easton. These ratios of the plant measurements thus express each average daily value for Oakland in terms of the corresponding average for Easton, and bring out the relations between the climatic conditions for the two stations, as indicated by the culture plants.

The daily averages of the various weather data for the entire experimental season are also given, in columns 7 and 8 of table XIII, only one of the three temperature efficiency indices (the one corresponding to the daily mean, tables X and XII, lines 10) being given here. Column 9 gives the ratios obtained by dividing each climatic value for Oakland by the corresponding value for Easton. Thus, these climatic ratios express each one of the various climatic values for Oakland in terms of the corresponding one for Easton, as in the case of the growth values.

The most evident feature brought out by the plant data given in table XIII is that the values for Easton are generally greater than the corresponding ones for Oakland. This is strictly true of the values based on the shorter observation periods (plants about 2 weeks old from seed) and it is true of those based on the full culture periods (plants about 4 weeks old from seed) excepting in the case of the mean daily rate of increase in the total number of leaves per plant and in that of average length of mature leaves. The greatest difference between the average daily growth rates for the two stations occurs in the case of the rate of increase in the average of the products of leaf length by leaf width (leaf-product,  $P$ ) for the shorter periods. For these younger plants the ratio of this rate for Oakland to that for Easton is 0.73. The greatest difference in these values based upon plants about 4 weeks old, for the two stations, is shown by the rates of increase in leaf area, dry weight of tops, and leaf-product, the ratios of these rates for Oakland to those for Easton being 0.80, 0.86 and 0.89, respectively. The rate of increase in leaf area, measured either directly (by the planimeter) or approximately (by means of the leaf-product), and also the rate of increase in dry weight of tops, since these rates exhibit the greatest differences between the two stations, may be considered as of probable value for comparing the effectiveness of the climatic conditions for the growth of the culture plants at one station with their effectiveness at the other.

The differences in the average daily values obtained for the climatic conditions, as here recorded for the two stations (table XIII, columns 6 to 9), are quite as great as or greater than the differences in the growth values. In comparing these it is to be remembered that the averages of the climatic data for the 2-week and 4-week periods are both computed from the same series of measurements, covering practically the same periods of time (these periods corresponding to the periods for which the plant data are computed), so that the average values derived from the two lengths of period are very nearly the same in this case, and they may be generally considered as practically identical for the purpose of comparison with the daily averages of the plant data for the two stations. The daily averages of effective temperature (temperature in excess of 40° F.) and of the efficiency index (derived from the chemical coefficient) are all about 84 per cent. as great for Oakland as they are for Easton, and a similar relation holds for the mean daily rate of evaporation, this rate being about 88 per cent. as great for Oakland as for Easton. The mean daily rainfall on the contrary is about 80 per cent. *greater* for Oakland than for Easton, and the rainfall-evaporation ratio for Oakland is more than double that for the other station. Similarly, the average daily duration of sunshine is about 43 per cent. *greater* for Oakland. Finally, the Easton station shows a higher average daily mean temperature, by about 5°F., but a smaller average daily range of temperature by about the same amount.

It appears that the average of the climatic conditions experienced by the successive cultures at Easton was more favorable for increase in leaf surface and in dry weight of tops (for both the 2-week and the 4-week periods) than was the corresponding average experienced by the cultures at Oakland. Excepting the average daily mean temperature, all of the major groups of environmental factors here considered were very different for the two stations, and the daily averages given in table XIII do not indicate which factor, or group of factors, may have been most influential in bringing about these differences in the growth rates. Of course it is possible that other factors than those considered in this study may also have been influential in producing the recorded differences in plant growth here brought out. Some information as to the relative influences exerted by the different groups of climatic conditions that have been instrumentally measured will appear below.

TABLE XIII

Seasonal averages of plant growth and of climatic conditions for Oakland and for Easton

DATA OF PLANT GROWTH				DATA OF CLIMATIC CONDITIONS			
	For Oakland (A)	For Easton (E)	For Oakland in terms of corresponding value for Easton $\left(\frac{A}{E}\right)$		For Oakland (A)	For Easton (E)	For Oakland in terms of corresponding value for Easton $\left(\frac{A}{E}\right)$
Average daily rate of increase in stem height, <i>cm.</i>	0.30	0.34	0.88	Average daily mean temperature, <i>degrees F.</i>	65.0	68.9	0.94
Average daily rate of increase in total no. of leaves per plant	0.14	0.16	0.88	Average daily mean effective temperature (above 40° F.), <i>degrees F.</i>	25.0	28.9	0.87
Average length of mature leaves ( <i>l.</i> ), <i>cm.</i>	2.4	3.1	0.77	Mean daily range of temperature, <i>degrees F.</i>	27.1	20.1	1.35
Average width of mature leaves ( <i>w.</i> ), <i>cm.</i>	2.1	2.4	0.88	Average daily temperature efficiency index (by chemical coefficient, from average daily means)	2.62	3.11	0.84
Average daily rate of increase in leaf-product, ( <i>P</i> ),	0.86	1.18	0.73	Mean daily rainfall ( <i>P</i> ), <i>cm.</i>	0.33	0.18	1.83
				Mean daily evaporation from cylindrical porous cup atmometer ( <i>E</i> ), <i>cm.</i>	15.5	17.5	0.89
				Average rainfall-evaporation ratio $\left(\frac{R}{E}\right)$	0.023	0.011	2.09
				Average daily duration of sunshine, <i>hours.</i>	8.5	5.9	1.45

Data for the shorter periods, representing approximately the first two weeks of growth from the seed.

Data for the longer period of 5, representing approximately the first four weeks of growth from the seed	Average daily rate of increase in stem height, <i>cm.</i>	0.23	0.24	0.96	Average daily mean temperature, <i>degrees F.</i>	65.3	69.9	0.93
	Average daily rate of increase in total no. of leaves per plant	0.12	0.11	1.09	Average daily mean effective temperature (above 40°F.), <i>degrees F.</i>	25.3	29.9	0.85
	Average length of mature leaves ( <i>l</i> ), <i>cm.</i>	2.9	2.9	1.00	Mean daily range of temperature, <i>degrees F.</i>	27.0	19.9	1.36
	Average width of mature leaves ( <i>w</i> ), <i>cm.</i>	2.1	2.2	0.95	Average daily temperature efficiency index (by chemical coefficient, from average daily means)	2.66	3.24	0.82
	Average daily rate of increase in leaf-product ( <i>P</i> )	1.17	1.32	0.89	Mean daily rainfall ( <i>R</i> ), <i>cm.</i>	0.34	0.19	1.79
	Average daily rate of increase in total leaf area per plant, <i>sq. cm.</i>	0.86	1.07	0.80	Mean daily evaporation from cylindrical porous cup atmometer ( <i>E</i> ), <i>cc.</i>	15.2	17.5	0.87
					Average rainfall-evaporation ratio ( $\frac{R}{E}$ )	0.024	0.011	2.18
	Average daily rate of increase in dry weight of tops per plant, <i>grams</i>	0.0044	0.0051	0.86	Average daily duration sunshine, <i>hours</i>	8.5 <sup>a</sup>	6.0 <sup>b</sup>	1.42

For Elkins, W. Va.

<sup>b</sup> Average of data for Baltimore and Washington.

# THE SEASONAL MARCH OF THE GROWTH RATES AND OF THE CLIMATIC CONDITIONS AT OAKLAND AND AT EASTON

## INTRODUCTION

Any season in any locality may be considered as made up of a series of relatively favorable and relatively unfavorable periods, all of which contribute to the sum total of the season's influence upon the magnitude of the final yield of plants. The ultimate influence of any given portion or period of the growing season upon the final yield cannot generally be judged by the appearance of the plants at any time before the yield may actually be measured, for the thrift and rate of growth of a plant during any period of its development is a result of both present and past conditions. Thus, the rapid growth of a crop observed during a hot, dry week in midsummer, after a rain that terminated a drought period, is partly a result of the rain, which supplied water for the expansion of the tissues, but it is probably almost as much due to conditions effective within the drought period, during which the plants may have elaborated reserve materials, an abundance of which is quite as requisite for rapid growth as is an abundance of water.

It is therefore necessary to study the seasonal march of each growth index and of each climatic index, as these vary throughout the season, for each station. These seasonal marches may be studied by employing the data recorded for either the two-week or the four-week periods; that is, by taking the first two weeks or the first four weeks of growth from the seed as the short period of observation for the plants. Since these two methods do not bring out the same points, both will be employed below. The following sections are devoted to a study of the seasonal marches of the various plant and climatic indices, (1) based on the two-week periods, and (2) based on the four-week periods.

## SEASONAL MARCHES BASED ON PERIODS OF APPROXIMATELY TWO WEEKS

*Plant growth rates.* Inspection of tables I and III brings out the fact that the three kinds of leaf measurements (length and width of mature leaves and leaf-product) nearly agree in the direction of their variation from period to period for both stations. On this account only one of these leaf measurements, the leaf-product, which shows the greatest variations, will be studied here.

On the whole it appears that, of the five criteria of plant growth here employed, those of leaf-product and stem height show all of the essential points, and seem to be sufficient to exhibit the differences in growth rates from one period to the next. These alone will therefore receive attention.

The variations in these two growth rates, from period to period, may be

best represented by means of the graphs of figure 8 which give the average daily values of leaf product and stem height for Easton and for Oakland. The ordinates of the points on these graphs are the successive daily averages per period of about two weeks, and the abscissas represent time and season, indicating the ends of the successive periods. Each graph thus shows the values of a single growth index as it varies through the season. The actual duration of the period for which any ordinate represents the average rate is indicated on the upper graph in each case (representing leaf

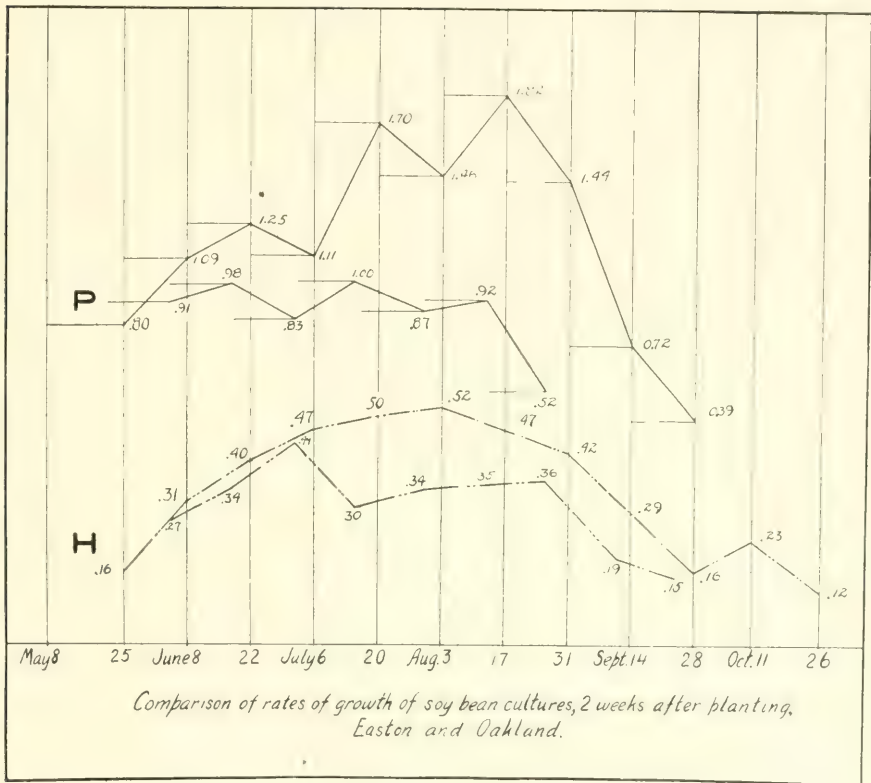


FIG. 8. *P*, leaf-product; *H*, stem height. The upper graph represents Easton, in each case.

product for Easton and for Oakland, respectively) by the length of the horizontal line extending to the left of the given point. The points on the corresponding graphs for stem height for Easton and for Oakland represent averages for the same time periods as are shown on the graphs of leaf-product.

Each of these graphs exhibits an evident seasonal march, the daily average per period increasing during a part of the season and then decreasing to low autumn values. This variation is more pronounced for Easton than for Oakland in both cases, which suggests that the seasonal changes in the

environmental conditions were more pronounced at the former station. There is no apparent similarity of detail, however, between the seasonal marches of the two graphs for either leaf-product or stem height. The maximum in leaf-product for Easton occurs for the period from August 3 to 17, while that for Oakland occurs two weeks earlier. It must be noted, however, that a secondary maximum for Oakland occurs for the period from June 18 to July 2, and that this secondary maximum is almost as great as the other. It thus appears that the maximum rate of increase in leaf area may be expected to occur considerably earlier at Oakland than at Easton. In a similar manner the maximum rate of increase in height for Oakland is seen to occur about a month earlier than that for Easton.

This apparent difference in the seasonal position of the optimum period for the growth of these plants at the two stations, if it should prove to be a normal occurrence and if it be a characteristic difference between lowland and mountain (or inland and coastal) localities for this region, might suggest interesting explanations of various agricultural facts; for example, the common observation that grains and other crops ripen earlier at high altitudes than at lower ones. If the progress of the life cycles of such crops should be associated with the seasonal march of what might be termed the plant-producing power of the climate, and if the decline that follows the attainment of the maximum in the environmental tendency to produce vegetative growth should prove to be important in accelerating flower and fruit production, (both of which suppositions are quite possible, as far as is known at present), then the normal date at which this optimum is reached in any locality might be of great significance in determining the tendency of the climate of that region to produce early maturation of agricultural plants. Other more or less similar considerations might be mentioned, but no such questions as this can be answered without extensive investigation, continued through several years.

*Climatic conditions.* The three sets of daily climatic data available for this study of the two-week periods (those of temperature conditions, those of moisture conditions, and those of light conditions), as given in tables IX and XI, are set forth graphically in figures 9 and 10, the former referring to Oakland and the latter to Easton. To facilitate comparisons between the seasonal marches of these climatic averages and the corresponding marches of the two sets of values for average daily rates of plant growth for the first two weeks from the seed, the graphs of figure 8 are repeated in figures 9 and 10. Thus each of the latter figures comprises five graphs, all referring to the same station and to the same series of two-week periods. The upper three refer to climatic conditions and the lower two to the corresponding rates of plant growth. These five graphs are comparable, in each case, as to direction of slope and as to the position of minima and maxima, but it should be remarked that the lengths of their ordinates are not directly com-

parable; the vertical scales employed are simply convenient ones and are quite arbitrary.

The graphs of temperature efficiency ( $T$ ) exhibit comparatively regular seasonal marches in both figures. The ordinate value of this graph for Oakland (figure 9) increases from 24.3, for the second two-week period, to 27.6 for the third period, continues high during periods 4 and 5, and then decreases steadily to a final value of 20.7 for the ninth period. The ordinate value of the corresponding graph of effective temperature for Easton (figure 10) continues to increase for a longer time in the early part of the season. It attains a maximum value of 37.0 for the fifth period (which roughly corresponds to the fourth period at the other station), remains comparatively high (about 35.0) until the beginning of the ninth period (September 1), and then falls quite rapidly to a minimum of 12.8 for the thirteenth period, the last of the season. Thus the highest temperature efficiencies for Oakland occurred about two or three weeks earlier than those for Easton, and the final decline of this climatic condition began a month earlier at Oakland than at the other station.

Another marked difference between the graphs of effective temperature for these two stations lies in the actual magnitudes of the various values. Not only are the values for corresponding time periods lower for Oakland than for Easton, but the differences between the highest and lowest value for Oakland is markedly smaller than the corresponding difference for Easton. The highest average effective temperature for Oakland is 27.7, while the highest for Easton is 37.0, and the minimum value for Oakland is 20.7, while the minimum for Easton is 12.8. The minimum value for Easton, however, occurred about a month after the Oakland season had been terminated by killing frost. From the above comparisons it appears that, in spite of the greater diurnal and interdiurnal variations in temperature recorded for Oakland, the seasonal march of the mean daily effective temperature here shows smaller differences between the extremes for the season than are exhibited for Easton.

The graphs for sunshine ( $S$ ) show a general downward slope throughout the season, for both stations, but both of the graphs are very irregular.

The graphs for the rainfall-evaporation ratio  $\left(\frac{R}{E}\right)$  are also irregular for both stations. Both show low values for the beginning and end of the season. That for Oakland shows a maximum for the two-week period ending July 30, and another maximum for the two periods ending August 26 and September 11. The maxima should indicate periods of most favorable moisture conditions and the minima should indicate those of relative drought. The Easton graph for this ratio also shows two maxima (one for the period ending July 6 and the other for that ending August 31) but these do not correspond, in the periods of their occurrence, with the maxima shown for

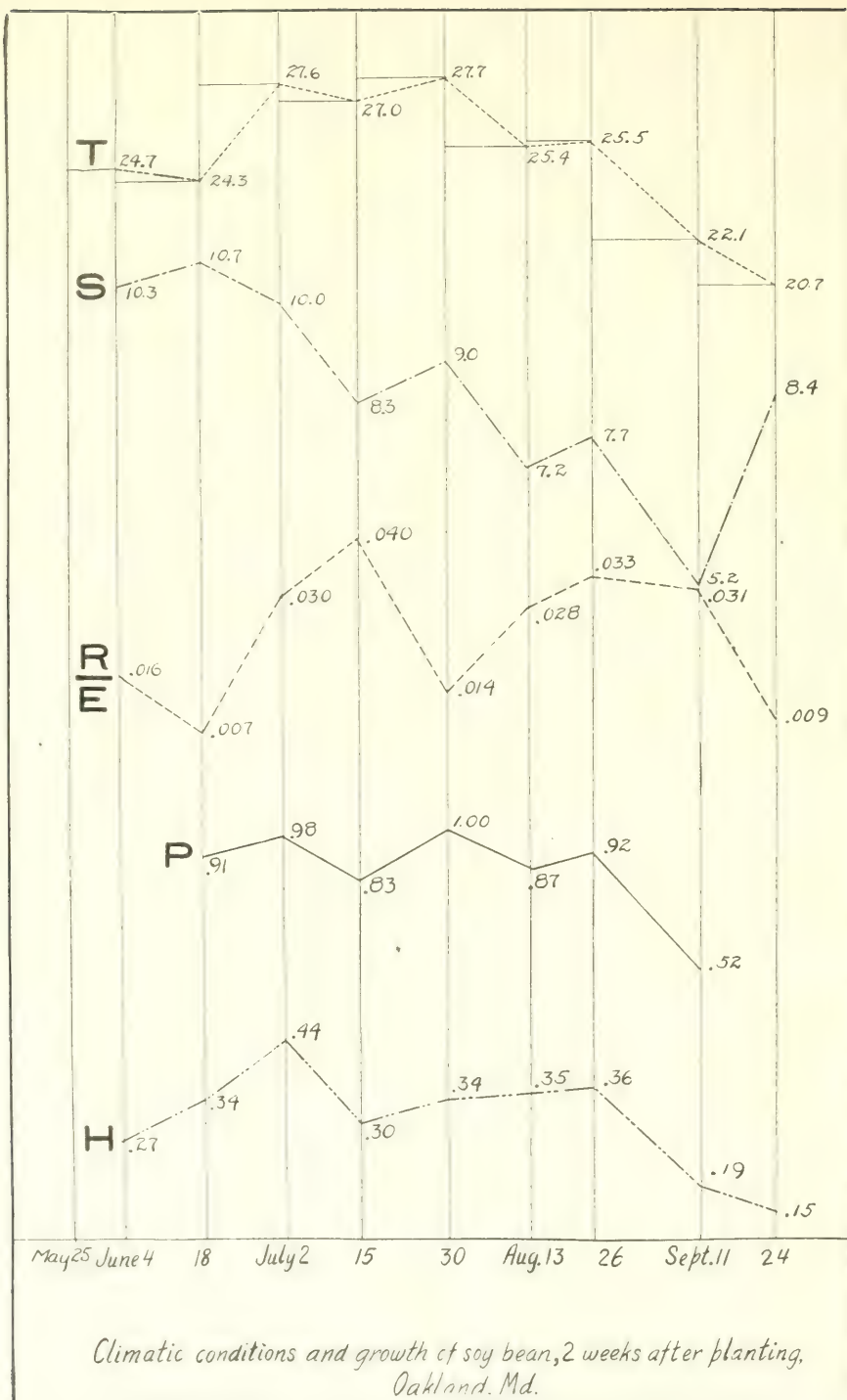


FIG. 9. T, temperature efficiency; S, sunshine duration;  $\frac{R}{E}$ , rainfall-evaporation ratio; P, leaf-product; H, stem height

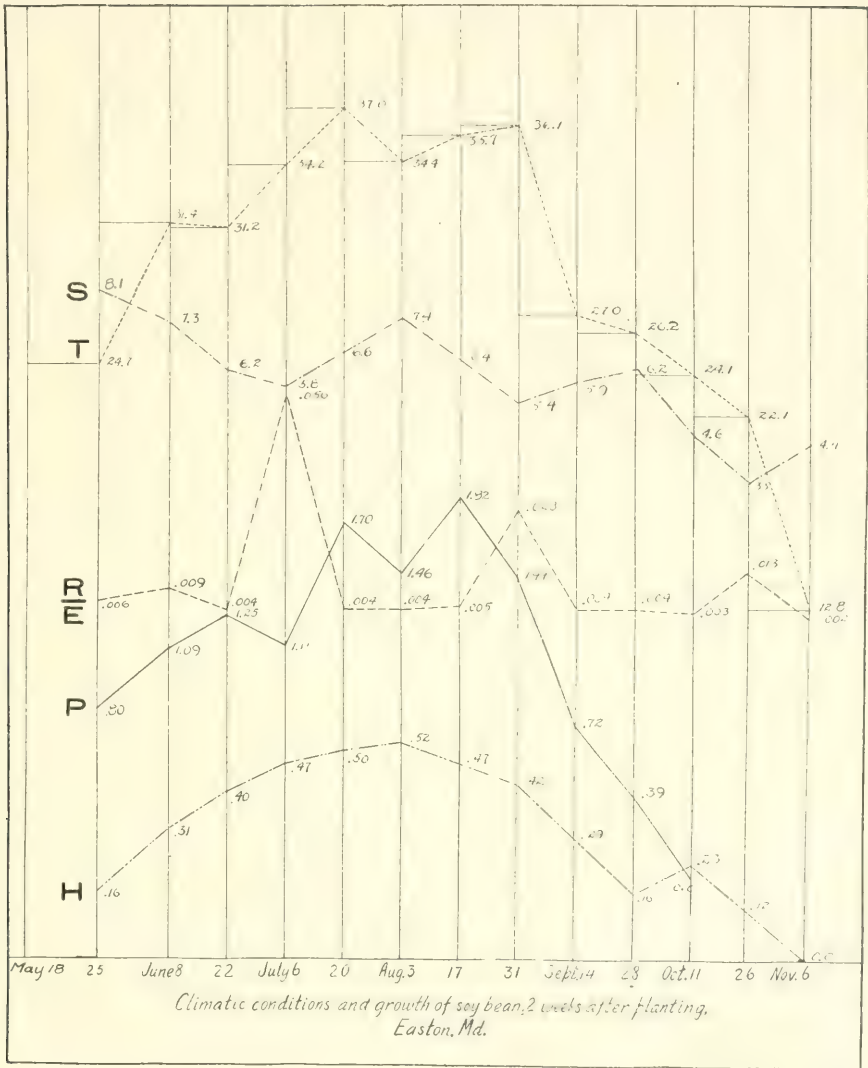


FIG. 10.  $T$ , temperature efficiency;  $S$ , sunshine duration;  $\frac{R}{E}$ , rainfall-evaporation ratio;  $P$ , leaf-product;  $H$ , stem height

Oakland, and the Easton graph is otherwise generally nearly horizontal. It is noteworthy that the long-continued low values of this moisture ratio for Easton are markedly lower than the lowest values for the other station, also that the first maximum value for Easton is higher than either maximum for Oakland. It thus appears that the moisture conditions at Oakland were generally more favorable than those at Easton but that they were subject to greater extremes of fluctuation at the latter place. An interesting point is brought out when the graph for the moisture ratio is compared with that for sunshine duration, for the same station, in that a strong correspondence exists between these two graphs. Low values of the rainfall-evaporation ratio correspond generally to high magnitudes of the sunshine value, and conversely, for both stations. The moisture graph appears strikingly like what the sunshine graph would be if it were inverted. On days of prolonged sunshine the rainfall influence was low and the evaporation influence was high.

*Comparison of graphs of growth rates with those of climatic conditions, for Oakland and for Easton.* The graph of daily increase in leaf-product for Oakland (fig. 9) shows the same direction of slope as does the one for average daily mean effective temperature, from point to point throughout the season. To the extent to which the sunshine graph agrees in direction of slope, with that for temperature, it also shows the same directions of slope as do the corresponding sections of the graph for leaf-product. There appears to be no relation between the graph of leaf-product and that of the rainfall-evaporation ratio for Oakland. The graph of the average daily increase in height is similar to that of leaf-product for all periods except the sixth (ending Aug. 13). For this period the graph of leaf-product shows a marked decrease from the previous period, while that for stem height increases slightly. Here the graph for leaf-product follows the direction of the temperature efficiency graph, as in the case of the other periods.

The graphs of effective temperature, of leaf-product and of stem height, for Easton (fig. 10), also show a general similarity in their seasonal marches. The sunshine and effective temperature graphs do not agree in trend for this station, as they do to a considerable extent for Oakland, and the graphs of growth rates show little tendency to conform to the variations in the direction of the sunshine graph. It may be noted here that the pronounced downward slope of the temperature graph does not begin until after the beginning of the corresponding decline in the graph for leaf-product. The temperature value continues high for about two weeks after the value of the leaf-product begins its downward trend. The decline in the graph of stem height begins even earlier than that in the graph of leaf-product.

In conclusion, it may be said that both of the graphs of growth rate for soy-bean seedlings within the first two weeks after planting exhibit distinct seasonal marches, which very closely resemble those of the corresponding

graphs for the temperature efficiency values. No apparent relation is noticeable between the two graphs of growth rates, on the one hand, and those representing the other two climatic factors, on the other. During the first two weeks after planting, the seedlings germinated and their stems elongated rapidly. The first pair of leaves were formed, but usually were not fully developed, at the time of the first measurement (approximately two weeks after planting). Thus, growth during this early period consisted largely in stem elongation, and seems in these experiments to have been most influenced by temperature. Since most of this development must have been accomplished at the expense of stored materials in the seed, and since an abundance of soil moisture was at all times supplied by the auto-irrigators, the principal factors affecting the rate of development of the plants were probably the rate of hydrolysis of stored materials and the rate of conduction of the latter from the cotyledons to the growing points. Since such processes, and also those of growth itself, are profoundly influenced by temperature, it is not surprising that correlations with temperature conditions are the only ones brought out by these graphs. It is not to be implied, however, that temperature was here the only influential factor, although it appears to be the most influential one, as far as these studies show.

#### SEASONAL MARCHES BASED ON PERIODS OF APPROXIMATELY FOUR WEEKS

*Plant growth rates.* As previously stated, each of the cultures was continued for a second period of two weeks, at the end of which time the plants were again measured. The behavior of the plants during this latter period of two weeks was somewhat different from that during the first period. The leaves expanded much more rapidly during the second fortnight than during the first, so that a considerable leaf surface was finally developed. In most instances the cotyledons remained attached to the plants throughout the entire month of growth, but they became yellow in many cases and probably usually became practically devoid of stored materials by the end of the month; in a few instances they fell from the plants before the time to harvest. Thus, with altered form and somewhat modified nutrition, the conditions influencing the rate of development of the plants might be expected to produce different results in the second two-week period from those produced in the first.

The most important daily increments obtained from these final measurements (tables 2 and 4) are shown graphically in figures 11 and 12, which are similar to figures 9 and 10. Two additional sets of measurements, not available for the two-week periods, were taken at this final observation, those of actual leaf area and those of dry weight. The data of leaf area, obtained from photographic prints, is to be regarded as a true measure of the extent of the leaf surface at the end of the four-week period. The leaf-

product was also obtained for the four-week periods, and, as has already been shown, these two measures of leaf area have a nearly constant ratio to each other, so that the relative rates of increase in leaf surface for the same plants during the two lengths of observation period may be directly compared by means of the leaf-product. Therefore the graphs showing rates of increase in leaf-product are given in figures 11 and 12, and the graphs of actual areal increase are not presented.

The data of dry weight of tops are especially important, since these are the only values obtained that furnish information on the approximate daily rates of accumulation of non-aqueous materials in the plants, and the graphs of average daily rate of increase in dry weight are included in figures 11 and 12.

In the case of Oakland the graphs of daily increase in leaf length, leaf width and total number of leaves, for these later measurements, were found to show a seasonal trend similar to that exhibited by the leaf-product, and they showed little variation in this respect for Easton. Hence these three criteria are not shown by the graphs of figures 11 and 12, and the graphs for daily increase in dry weight of tops, leaf-product and stem height are the only ones referring to plant growth given in these figures.

Each of the graphs of average daily growth rates for the four-week periods for Oakland (fig. 11), as well as each one for Easton (fig. 12), exhibits a seasonal march similar to that shown by the corresponding graph for the two-week periods. These three four-week graphs are similar in form for Oakland, being generally convex upward, but they all show concavity upward in the region of the fourth and fifth periods, this concavity being only slightly evident in the case of the graph for rate of increase in dry weight. The latter rises to its maximum for the third four-week period and then descends to the end of the season, with but a slight rise for the period ending August 13, while each of the other two shows two maxima.

For Easton, the graphs of increase in dry weight and in leaf-product agree with the one of increase in leaf-product for Oakland in showing two maxima, with a concavity between them, and the graph of increase in dry weight for Oakland shows the first of these maxima, but the dates of these maxima are not the same for the two stations. While these two periods giving low values of the leaf-product for Oakland were those ending July 30 and August 13, the corresponding low values for Easton (of both weight and product) are for the periods ending July 20 and August 3. There thus appears to have been a difference of ten days in the dates corresponding to these low values of the graphs, the upward concavity occurring earlier at Easton. In this connection it must be remembered, however, that records were obtained only at approximately two-week intervals, so that the exact dates corresponding to these two low values, or to the two maxima to which they are related, cannot be accurately fixed by the data at hand.

The graph of average daily increase in stem height for Easton shows a single maximum near the center of the season, for the sixth period.

*Climatic conditions.* As has already been noted, the weather data for the four-week periods corresponding to the duration of the cultures represented in the final measurements of the plants, are all derived from the data for the two week periods, to which they are naturally similar. The graphs of the three average daily values used for the fortnight periods (temperature, sunshine and rainfall-evaporation ratio) are shown for the longer periods in figures 11 and 12, these three graphs being again comparable as to direction of slope and position of maxima and minima, but not as to the actual heights of their ordinates. The temperature graphs are of course smoother in this case than in that of the two-week data. The converse correspondence between the graph of the moisture ratio and that of sunshine duration is not as striking here as for the two-week periods, but is nevertheless apparent.

*Graphs of growth rates compared with those of climatic conditions, for Oakland and for Easton.* The graphs of plant growth (figs. 11 and 12) show no apparent relation to the marches of either rainfall or evaporation alone, but they do exhibit some interesting agreements with the march of the rainfall-evaporation ratio, which is graphically shown in these figures. While several periods for Easton show low sunshine values as concomitant with high values of leaf-product and dry weight, and while one such correspondence is evident in the case of Oakland, yet the failure of this relation to be general and the somewhat unsatisfactory nature of the sunshine data render the relation itself somewhat questionable. Of course it may sometimes occur that too strong sunshine may retard plant growth through the moisture relation, and thus bring about such a correspondence as that just mentioned. The rainfall-evaporation ratio (representing the moisture relation) will receive attention below.

For the data obtained two weeks after planting (figs. 9 and 10) there is no obvious agreement between the seasonal trend of the plant measurements and that of either sunshine duration or rainfall-evaporation ratio, but there is a general agreement between the seasonal march of effective temperature ( $T$ ), and that of stem height ( $H$ ) for both stations, as has been pointed out. Similarly, the graph of leaf-product ( $P$ ) for Easton shows a pronounced parallelism to that of effective temperature, but this agreement is not shown for Oakland. It thus appears that the temperature conditions were the main controlling factor in the first two weeks of stem elongation for both Oakland and Easton, but that this temperature control was not precise, some influence being exerted by other factors. It also appears that leaf expansion during the first 2 weeks of growth (measured by leaf-product) was rather definitely controlled by temperature conditions at the Easton station, but that other factors were not without influence upon this process at this station, these other factors constituting the main control at Oakland.

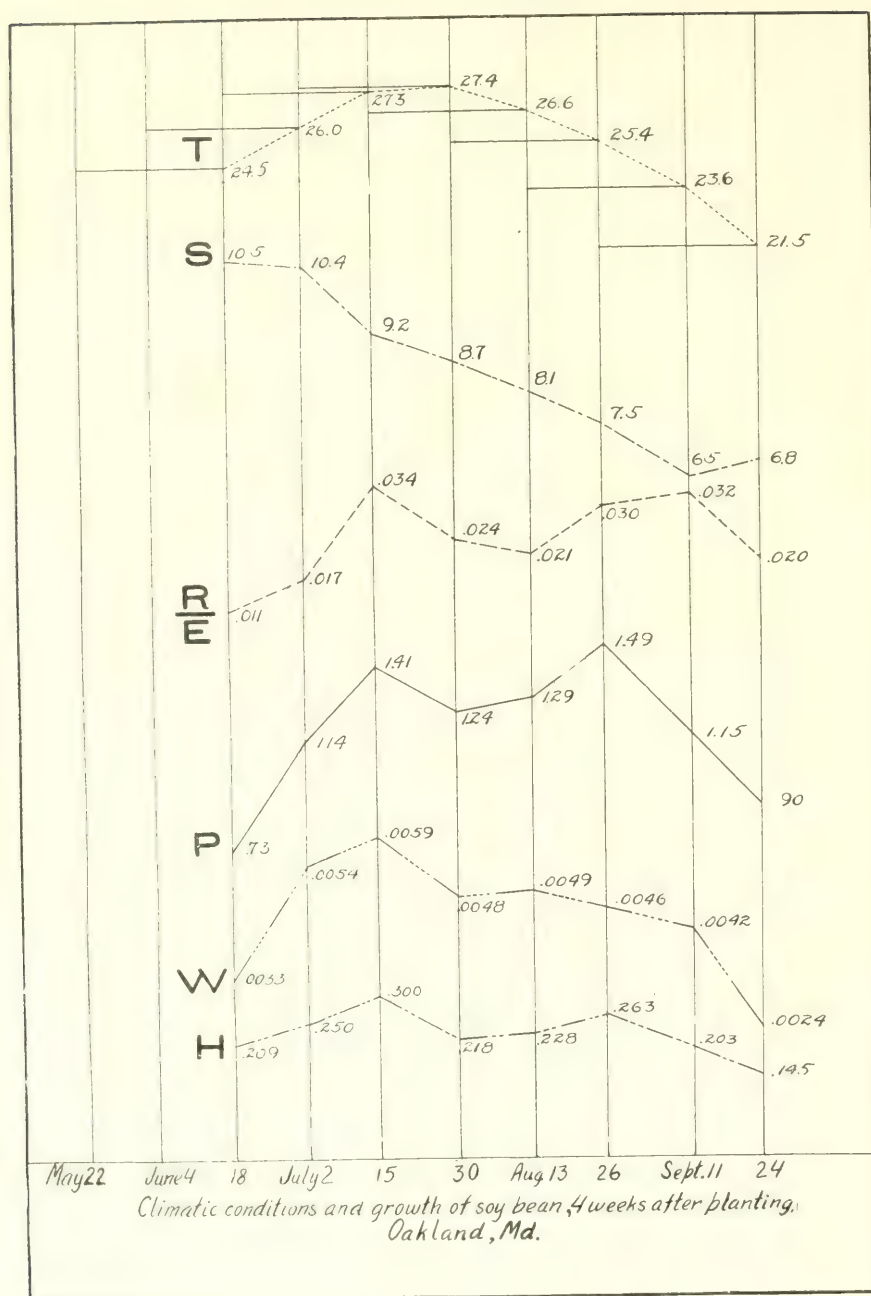


FIG. 11.  $T$ , temperature efficiency;  $S$ , sunshine duration;  $\frac{R}{E}$ , rainfall-evaporation ratio;  $P$ , leaf-product;  $W$ , dry weight;  $H$ , stem height

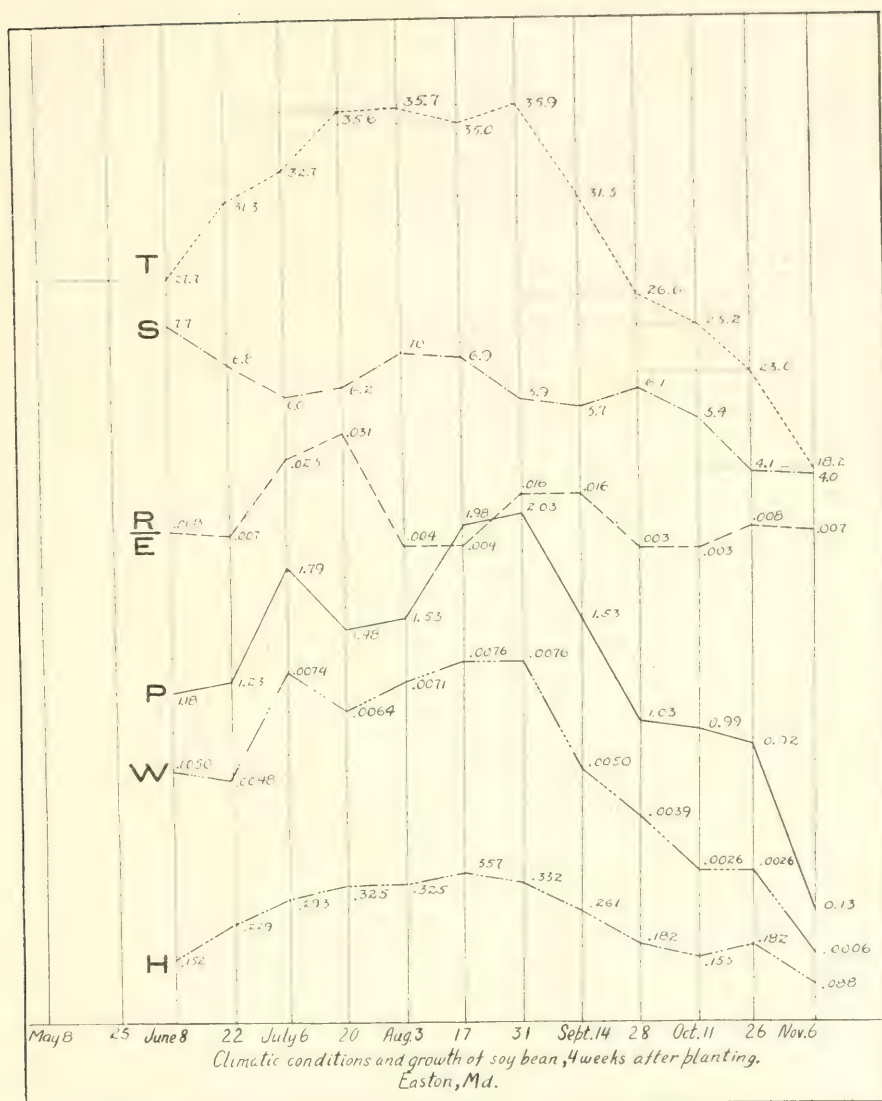


FIG. 12.  $T$ , temperature efficiency;  $S$ , sunshine duration;  $\frac{R}{E}$ , rainfall-evaporation ratio;  $P$ , leaf-product;  $W$ , dry weight;  $H$ , stem height

In the case of the data obtained 4 weeks after planting (figs. 11 and 12), the graph of temperature conditions shows a general similarity to all three plant graphs, for both stations, but this agreement is only general. The plant graphs for leaf-product ( $P$ ) and stem height ( $H$ ) for Oakland agree with each other in showing two maxima (periods ending July 15 and Aug. 26), and the first of these maxima is also shown by the graph of dry weight ( $W$ ). None of these maxima coincides in date with the single maximum of the graph of temperature efficiency. For Easton, the discrepancies of detail between the plant graphs and the the graph of temperature efficiency for the 4-week periods are fully as pronounced as those just mentioned. The graphs of leaf-product and weight both show two maxima (periods ending July 6 and Aug. 31), while this double maximum is barely indicated on the temperature graph. The graph of height fails to agree with the irregularities of either of the other two plant graphs and agrees only in its general seasonal march with the temperature graph. The four-week graph of sunshine shows no parallelism with any of the plant graphs in the case of either station.

A study of the four-week graphs for Oakland (fig. 11) brings out the fact that the two maxima of each of the graphs of leaf-product and stem height coincide in time of occurrence with high points on the graph of rainfall-evaporation ratio and the depression between the two maxima on these growth graphs is also seen on the moisture graph. The second maximum of the moisture graph occurs a fortnight later than in the case of the graphs of leaf-product and stem height, but the upward slope of the moisture graph is slight for this fortnight. Also, the single maximum of the weight graph coincides in time with the first maximum of the graph of the moisture ratio. It thus appears that it is only for the beginning and end of the season that the direction of slope of the four-week plant graphs is generally the same as that of the corresponding temperature graph; during the middle portion of the season the plant graphs show a strong tendency to follow the direction of the moisture graph, even where this graph differs radically from that of temperature. It may be said, for Oakland, that the four-week rates of increase in leaf-product and in stem height follow the four-week moisture ratio for that portion of the season when the four-week temperature efficiency value is above about 25.

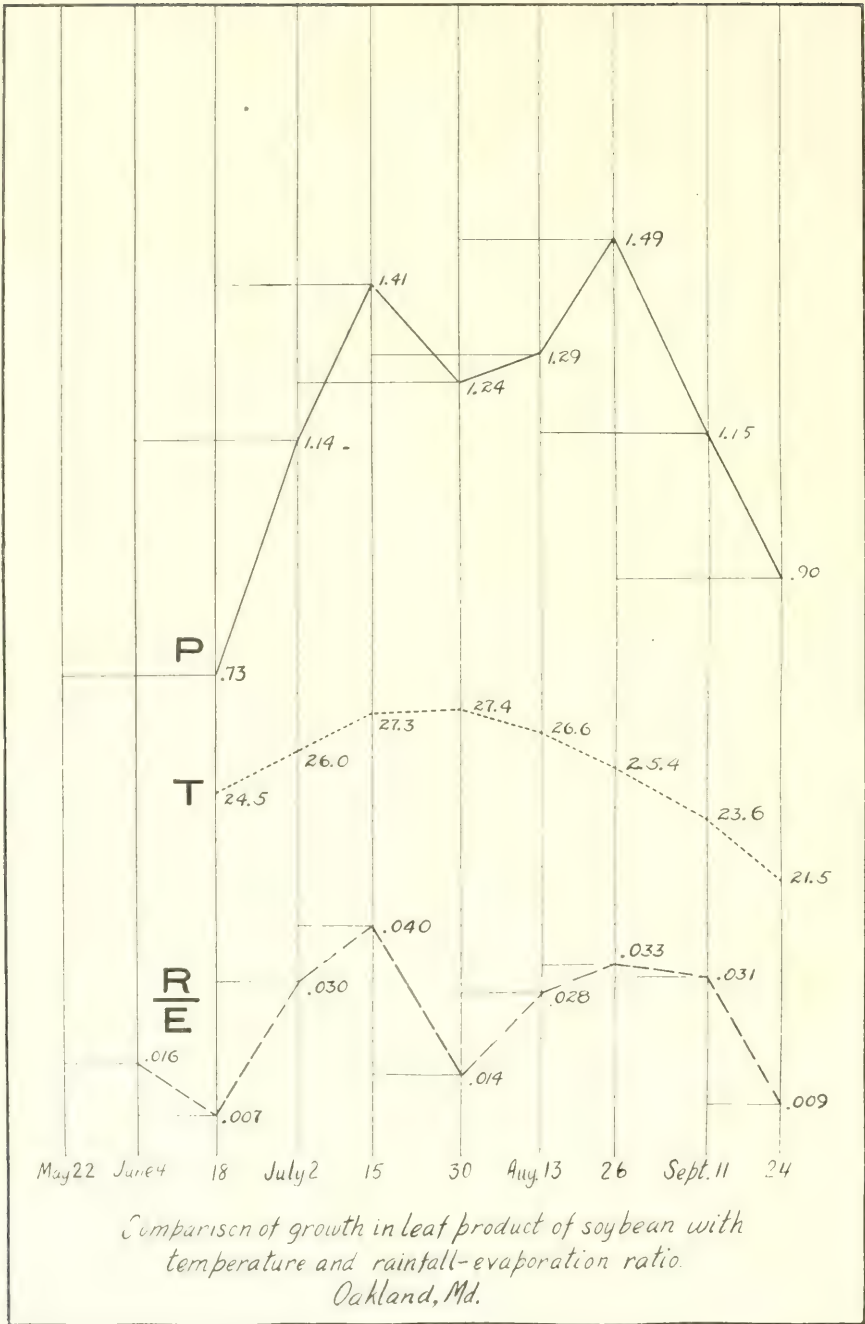
For Easton (fig. 12) the four-week data show a similar set of agreements and disagreements. In this case the height graph has nearly the same slope throughout as has the temperature graph, but the other two plant graphs show pronounced disagreements with the graph of temperature, excepting at the end of the season. It is suggested that these disagreements may be controlled by moisture conditions, but the two maxima of the four-week moisture graph do not synchronize with those of leaf-product and weight; the moisture ratio maxima occur a fortnight later. Here, again, it may be said that the plant graphs generally agree in direction of slope with the tem-

perature graph, from period to period, only when the temperature efficiency value is below about 25.

From the two-week and four-week graphs of figures 9-12 it may be tentatively concluded (1) that the temperature relation is the main controlling factor for the growth rates based on the 2-week periods, no suggestion being apparent as to just what factors besides temperature may have been influential; (2) that temperature conditions are the controlling or limiting factor for growth rates based on the four-week periods, as long as the temperature efficiency values are not above about 25; and (3) that the moisture conditions (represented by the rainfall-evaporation ratio) seem to have exerted a marked influence upon the four-week growth rates during the time when the temperature efficiency values were above about 25. From the fact that the influence of the moisture conditions is not apparent for the two-week data and is apparent or strongly suggested for the four-week data, it appears probable that the moisture relation is relatively more important in the later stages of the development of the plants than in the earlier ones. If this supposition be true it may explain why the growth rates tend to follow the fluctuations of the moisture ratio as the plants become older. In such a case it should be the moisture conditions of the *last 2 weeks* of each of the four-week periods that are influential in determining the average growth rates for the four-week periods. To test this supposition, the four-week data of leaf-product and the rainfall-evaporation ratios corresponding to the last two weeks of each four-week period have been brought together for comparison in the graphs of figures 13 and 14, the former for Oakland and the latter for Easton. In these figures the four-week leaf-product graph ( $P$ ) is the upper one, being reproduced from figure 11 or 12. The four-week temperature graph ( $T$ ) lies next below, also derived from figure 11 or 12. The third graph presents the two-week data of the rainfall-evaporation ratio  $\left(\frac{R}{E}\right)$  and is reproduced from figure 9 or 10. The length of period represented by each of the successive values of the leaf-product and of the moisture ratio is shown by a horizontal line extending to the left of each point on these graphs.

Figures 13 and 14 indicate a pronounced parallelism between the four-week graph of leaf-product and the 2-week graph of the moisture ratio (for the last half of the four-week period), for both stations, thus furnishing evidence in favor of the supposition set forth above. It appears that the general rate of growth of these plants was influenced by temperature throughout the entire four-week period, but that the rate of leaf expansion was definitely influenced also by the moisture relations effective during the *last 2 weeks* of that period.

It seems reasonable to suppose that as the plant grows larger its sensitiveness to changes in its moisture surroundings increases; the greater expanse



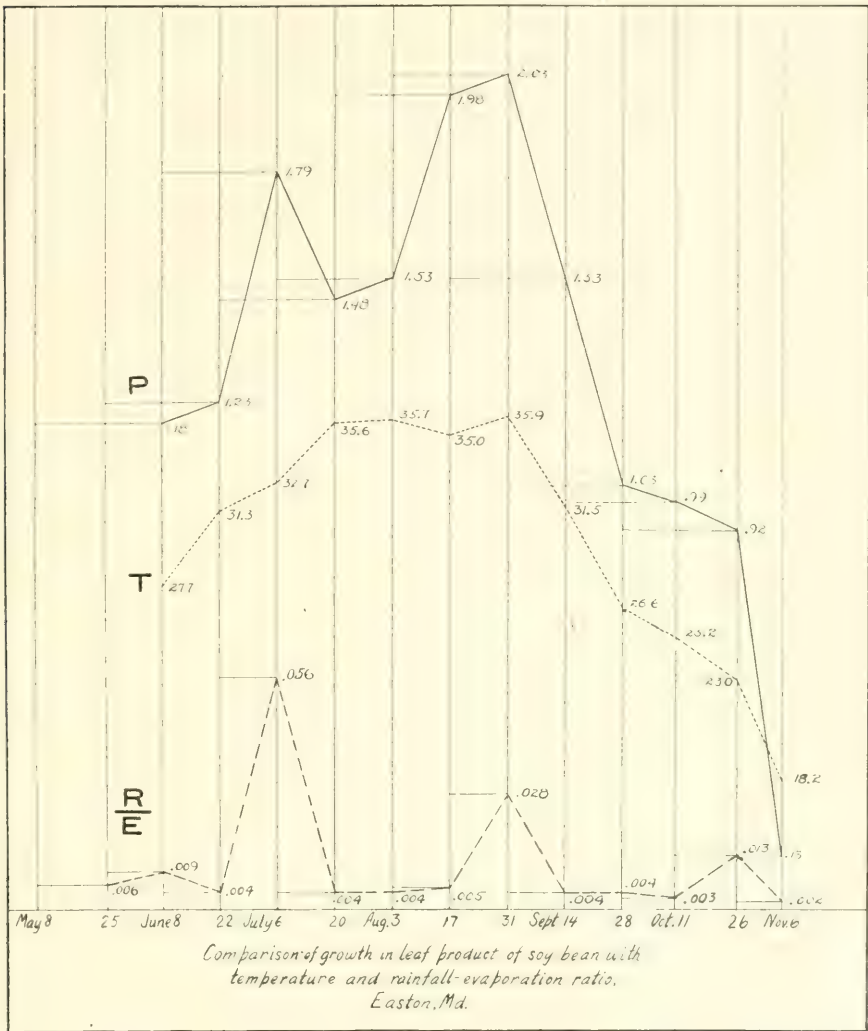


FIG. 14.  $P$ , leaf-product;  $T$ , temperature efficiency;  $\frac{R}{E}$ , rainfall-evaporation ratio

of surface should be accompanied by an increased requirement for water to supply that lost by transpiration. If this be granted it follows that the moisture conditions of the environment might be more than adequate for the early stages of development, while for later stages these same conditions might be limiting factors.<sup>32</sup> This condition of affairs is suggested by the relation just brought out, between the moisture ratio and the rate of increase in leaf expanse. These plants never experienced pronounced drought, for they were always abundantly supplied with soil moisture and the evaporation intensities of this region are never very high. There were slight fluctuations in the moisture conditions, however, and if these were to affect the plants at all this should occur when the requirement for water is greatest; namely, in the later stages of development, when a relatively large surface is exposed to the air and sunshine. Furthermore, since rapid transpiration tends to deplete the water-content of leaves more than that of stems, it follows that leaf-growth should be retarded more than stem-growth, as the plant begins to experience a moisture deficit. The data of the present studies indicate that the culture plants were not markedly limited by the moisture conditions during the first two weeks of their growth from the seed, but moisture conditions did limit leaf expansion in the second two-week period from the seed. The moisture conditions here varied but little, but they seem to have varied enough to influence the plants when the latter were most sensitive to them, and this influence became most markedly evident in that growth process (leaf expansion) which should be the most sensitive to these conditions. It may be added that this paramount influence of the moisture conditions would be expected to become evident especially in periods of high temperature, when transpiration should be most accelerated. With lower temperatures (efficiency values below about 25, according to these data) the moisture conditions should lose their power to influence the plant, and temperature should become the main controlling condition. This is in accord with the facts above brought out.

These various considerations may be summarized as follows. (1) Temperature exerts an influence at all times. (2) With low temperatures this influence is the controlling one, but with high temperatures the moisture relation becomes important. (3) When this occurs, it is the moisture conditions of the last 2 weeks that control the average rate of leaf expansion for a four-week period.

A response in the growth rate to either one of two sets of climatic conditions

<sup>32</sup> On the general theory of limiting conditions and its application, see the following:

Blakesman, F. F., Optima and limiting factors. *Ann. Bot.* **19**: 283-295. 1905.

Mitscherlich, E. A., Das Gesetz des Minimums und das Gesetz des abnehmenden Bodenartrags. *Landw. Jahrb.* **38**: 537-552. 1909. *Idem*, Ueber das Gesetz des Minimums und die auf diesem ergebenden Schlussfolgerungen. *Landw. Versuchsstat.* **75**: 231-263. 1911.

Smith, A. M., On the application of the theory of limiting factors to measurements and observations of growth in Ceylon. *Ann. Roy. Bot. Gard. Peraduniya* **3II**: 303-375. 1906.

within the same extreme limits of environmental conditions, such as is indicated in this case for the four-week periods, was somewhat similarly demonstrated by Smith (1906), for shorter observation periods, in a study of the relation of climatic conditions to the growth of the giant bamboo in Ceylon.

As has already been mentioned, the two-week data of sunshine duration correspond in an inverse manner to the data of the moisture conditions, and it follows that wherever the latter conditions appear to have controlled growth it also must appear that sunshine duration was an influential factor. The plants generally grew more with low sunshine values and less with high sunshine values. This somewhat unexpected observation seems to agree with the general physiological fact that plants actually grow most during the hours of darkness or of weak light, and it points clearly to the conclusion that the controlling influences of sunshine in these studies was exerted through the water relation. It has been suggested<sup>33</sup> that physiological retardation of growth by light is really largely due to increased transpiration during the daylight hours. If the sunlight influence noted above were exerted upon the photosynthetic process it would be expected to have the opposite direction to that actually indicated; the plants should grow *more* during periods having high sunshine values. But they are here found to grow less during such periods.

In this general connection it is to be remembered, however, that the sunshine data here employed are probably not as reliable as the other climatic data. Of course other conditions than sunshine duration are influential in determining the value of the moisture ratio, but the relation here brought out emphasizes the apparent importance of sunshine as a factor in the water relation of plants. This matter will repay serious study whenever adequate methods for measuring sunshine may become available.

## CONCLUSIONS

The study here reported was undertaken mainly to test, in a preliminary way, certain newly devised methods for attacking the general problem of the relation of plant growth to climatic conditions. The results given in the preceding pages show that some of these new methods are of value, and the data obtained by their means throw light upon the question of the influence and relative importance of several different climatic features, as these affected the growth of the culture plants.

The method here employed, of growing plants from seeds, as like as possible, in pots of like soil, for approximately equal short time periods at different stations, proved very satisfactory as a means of comparing climatic conditions for different localities and for different seasons of the year, as

---

<sup>33</sup> Palladin, W., Pflanzenphysiologie. Berlin, 1911. P. 257.

these conditions influence plant growth. Such culture plants may be regarded as integrating instruments for the measurement of climate. They are started from the resting condition, as seeds (in which state they may be considered as instruments set at the zero points of their scales), and the amount of growth accomplished after any given period of exposure may be taken as the summed result of all the environmental influences that have acted upon the plants during the period. Unlike many of the man-made contrivances employed as measuring instruments, the standard plants cannot be reset at the zero point after reading, but must needs be replaced by new and similar individuals in the resting stage. Errors due to individual variations in the plants, introduced by thus using a succession of standard plants, were not found to be excessive.

The method here employed for soil moisture control, employing auto-irrigators, proved very satisfactory for the purpose, although the details of this technique are susceptible of considerable improvement.

The methods by which the environmental conditions were measured in these studies were generally those in common use for similar purposes, and they require little comment here. The measurements of the evaporating power of the air, obtained with standardized cylindrical porous cup at mometers, taken with the ordinary precipitation records or measurements of rainfall gave a ratio of rainfall to evaporation that appears to be a very valuable measure of the environmental conditions as far as the water relation of the plants is concerned. Similarly, the records of daily maximum and minimum temperature readings, as obtained from maximum and minimum thermometers of the type now in common use by the U. S. Weather Bureau, were found to be entirely adequate for all the needs of temperature measurement encountered in this work. Concerning the sunshine records as here employed, obtained with the Marvin sunshine recorder, it appears that such data promise to be of some value in indicating the relative influence of sunshine duration in determining the magnitude of the rainfall-evaporation ratio, but no other relation between this climatic feature and the growth of the plants was discoverable.

Two methods of temperature summation were used in this study. One of these employs the direct summation of the daily mean temperatures above a certain assumed zero-point for growth, in this case above 40° F. The second is an indirect method, using temperature efficiencies derived from the application of the chemical principle of van't Hoff and Arrhenius, in place of the actual temperature readings above an assumed physiological zero. The two methods agree in showing a clear relation between temperature and plant growth at both Oakland and Easton, but no evidence was brought out as distinctly in favor of either of the two methods.

The general conclusions of this study, regarding the relation of temperature, moisture and light conditions to the growth of these soy bean seedlings,

have been summarized in the Abstract, at the beginning of the paper. While these conclusions constitute only a beginning, it appears that further study along lines similar to these may eventually develop a considerable knowledge of the relations here dealt with, and that this knowledge may furnish an important point of view for future climatological work. It promises, also, to be of practical value in connection with agriculture and forestry. In the planning of further studies, it must be borne in mind, however, that the problem is a very complex one, which cannot be expected to yield to any simple treatment. Nevertheless, it seems that climatology and plant physiology are now far enough advanced to warrant serious and sustained attack upon this very important question of the relation of climatic conditions to plant growth and development.

### LITERATURE CITED

- ABBE, C., First report on the relation between climate and crops. U. S. Weather Bur. Bull. 36. 1905.
- ARCTOWSKI, HENRYK, Studies on climate and crops: corn crops of the United States. Bull. Amer. Geog. Soc. 44: 745-760. 1912.
- BLACKMAN, F. F., Optima and limiting factors. Ann. Bot. 19: 283-295. 1905.
- BONSTEEL, J. A., The soils of Prince George's County. Maryland Geological Survey. Baltimore, 1911.
- BRIGGS, L. J., and H. L. SHANTZ, Daily transpiration during the normal growth period and its correlation with the weather. Jour. Agric. Res. 7: 155-212. 1916.
- BROWN, W. H., The relation of evaporation to the water content of the soil at the time of wilting. Plant World, 15: 121-134. 1912.
- COMBES, R., La détermination des intensités lumineuses optima par les végétaux au divers stades de développement. Ann. Sci. Nat. Bot. IX, 11: 74-254. 1910.
- FASSIG, O. L. The period of safe plant growth in Maryland and Delaware. Monthly Weather Rev. 42: 152-158. 1914.
- HAWKINS, LON A., The porous clay cup for automatic watering of plants. Plant World 13: 220-227. 1910.
- HILGARD, E. W., Soils, their formation, properties and composition. New York, 1911.
- JOHNSTON, E. S., and B. E. LIVINGSTON, Measurement of evaporation rates for short time intervals. Plant World 19: 136-140. 1916.
- KIESSELBACH, T. A., Transpiration as a factor in crop production. Nebraska Agric. Exp. Sta. Research Bull. 6. 1916.
- KIMBALL, H. H., The total radiation received on a horizontal surface from the sun and sky at Mount Weather. Monthly Weather Rev. 42: 474-487. 1914.
- KOEPPEN, W., Wärmezonen der Erde, nach der Dauer der heissen, gemässigten und kalten Zeit und nach der Wirkung der Wärme auf die organische Welt betrachtet. Meteorol. Zeitschr. 1: 215-226. 1884.
- LEHENBAUER, P. A., Growth of maize seedlings in relation to temperature. Physiol. Res. 1: 247-258. 1914.
- LIVINGSTON, B. E., A method for controlling plant moisture. Plant World 11: 39-40. 1908.
- , Light intensity and transpiration. Bot. Gaz. 52: 417-438. 1911.
- , A rotating table for standardizing porous cup atmometers. Plant World 15: 157-162. 1912.
- , Climatic areas of the United States as related to plant growth. Proc. Amer. Phil. Soc. 52: 257-275. 1913.
- , Atmometry and the porous cup atmometer. Plant World 18: 21-30, 51-74, 95-111, 143-149. 1915.
- LIVINGSTON, B. E., and LON A. HAWKINS, The water-relation between plant and soil. Carnegie Inst. Wash. Pub. 204: 3-48. 1915.
- LIVINGSTON, B. E., and G. J. LIVINGSTON, Temperature coefficients in plant geography and climatology. Bot. Gaz. 56: 346-375. 1913.
- LUBIMENKO, W., Production de la substance sèche et de la chlorophylle chez les végétaux supérieures aux différentes intensités lumineuses. Ann. Sci. Nat. Bot. IX, 7: 321-415. 1908.
- MACDOUGAL, D. T., The auxothermal integration of climatic complexes. Amer. Jour. Bot. 1: 186-193. 1914.
- MCLEAN, FORMAN T., Relation of climate to plant growth in Maryland. Monthly Weather Rev. 43: 65-72. 1915.
- MERRIAM, C. HART, Laws of temperature control of the geographic distribution of plants and animals. National Geog. Mag. 6: 229-238. 1894.
- MITSCHELICH, E. A., Das Gesetz des Minimums und das Gesetz des abnehmenden Bodenertrags. Landw. Jahrb. 33: 537-552. 1903.
- , Ueber das Gesetz des Minimums und die auf diesem ergebenden Schlussfolgerungen. Landw. Versuchsstat. 75: 231-263. 1911.

- PALLADIN, W., Pflanzenphysiologie. Berlin, 1911.
- PULLING, H. E., and B. E. LIVINGSTON, The water-supplying power of the soil as indicated by osmometers. Carnegie Inst. Wash. Pub. **204**: 49-84. 1916.
- RICHTER, A., Etude sur la photosynthèse et sur l'absorbtion par la feuille verte. Rev. gen. Bot. **14**: 151-169, 211-218. 1902.
- ROSE, E., L'énergie assimilatrice chez les plantes. Ann. Sci. Nat. Bot. IX, **17**: 1-110. 1913.
- SHIVE, J. W., An improved non-absorbing porous cup atmometer. Plant World **16**: 7-10. 1915.
- SHREVE, F., Rainfall as a determinat of soil moisture. Plant World **17**: 9-26. 1914.
- SMITH, A. M., On the application of the theory of limiting factors to measurements and observations of growth in Ceylon. Ann. Roy. Bot. Gard. Peradiniya **3II**: 303-375. 1906.
- SMITH, J. WARREN, The effect of weather upon the yield of corn. Monthly Weather Rev. **42**: 78-93. 1914.
- TRANSEAU, E. N., Forest centers of eastern North America. Amer. Nat. **39**: 875-889. 1905.
- , Apparatus for the study of comparative transpiration. Bot. Gaz. **52**: 54-60. 1911.
- ZON, R., Meteorological observations in connection with botanical geography, agriculture and forestry. Monthly Weather Rev. **42**: 217-223. 1914.

# THE INFLUENCE OF CERTAIN ENVIRONMENTAL CONDITIONS, ESPECIALLY WATER SUPPLY, UPON FORM AND STRUCTURE IN *RANUNCULUS*

DONALD FOLSOM

## ABSTRACT<sup>1</sup>

The aim of the experimentation described in this paper was the quantitative determination of the structural differences produced in *Ranunculus sceleratus* L. and *Ranunculus abortivus* L., by differences in the water supply during the period of development. The plants were grown with five different degrees of water supply, the conditions of five sets of cultures being characterized, respectively, by (1) submergence of soil and plant, (2) flooding of soil, (3) nearly constant capillary soil-water supply, (4) rather small soil-moisture content, and (5) only enough soil-moisture to support life. Some other environmental features were more or less modified artificially, but no attempt was made to control or measure any of the effective conditions of the surroundings excepting water supply. All experimental plants were grown in the same greenhouse. Various features of the roots, stems, leaves, flowers and seeds were examined and measured.

Three generations of *R. sceleratus* were grown, the first and second being largely alike as to the degrees of water supply used but differing markedly in regard to some of the non-water-supply conditions of the surroundings. Especially was the light weaker for the second generation than for the first, on account of shading the greenhouse by paint on the glass. The experiments of the third generation of this plant were planned to find out whether the effects of high or low water supply during the development of the parent might be evident in the offspring, but only one structural feature was studied, laminar area of the root leaves. One generation of *R. abortivus* was grown, during about the same period as that of the first generation of the other species.

By means of the average values for the plant features measured it is possible to make the following systematic comparisons. (a) Between plants of the same species grown with the same non-water-supply conditions but with different degrees of water supply. (b) Between plants of the same species grown with rather different non-water-supply conditions but with

---

<sup>1</sup> The manuscript of this paper was received April 13, 1918. This abstract was preprinted, without change from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 2, no. 5, November, 1918.

about the same degree of water supply. (c) Between the general water-supply relations of plants of *R. sceleratus* grown with the non-water-supply conditions of the first generation and the same relations as shown for the second generation of the same species. (d) Between different features of the same species when grown under each one of the tested complexes of environmental conditions. (e) Between the two species here studied, in regard to their general water-supply relations.

The following is a summary of the main results.

#### RANUNCULUS SCELERATUS

I. Plants of *Ranunculus sceleratus* grown with markedly different surroundings as to water supply, but not submerged, and otherwise all with the same set of environmental conditions, showed differences, in the following features, that were *directly* and consistently related to the water supply; for each of these features progressively lower water supply gave progressively smaller values. (1) Total height of plant. (2) Stem height to first flower. (3) Basal and median stem diameter. (4) \*Thickness of stem cortex. (5) \*Thickness of stem aerenchyma (both absolute and relative to cortex thickness). (6) Laminar area of root-leaves.

The same kind of direct relation to water supply is partially indicated or suggested (but not distinctly and consistently so in the results of these experiments) for the following structural features. (1) Root radius. (2) Thickness of root aerenchyma (both absolute and in terms of root radius). (3) Bundle interval of stem, absolute. (4) Petiolar width, root-leaves. (5) Number of petiolar bundles, root-leaves. (6) Laminar area of lowest stem-leaves. (7) Stomatal frequency, upper stem-leaves, lower surface. (8) Number of layers of chlorenchyma, root-leaves, lowest stem-leaves, upper stem-leaves. (9) Thickness of chlorenchyma, root-leaves. (10) Number of achenes per flower, first and tenth flowers to open. (11) Seed viability.

For just one structural feature is an *inverse* relation to water supply consistently shown; this feature is the bundle interval of the stem, when measured in terms of stem cortex thickness.

For the following structural features an *inverse* relation to water supply is more or less inconsistently or partially indicated. (1) Bundle interval of stem, when measured in terms of stem diameter. (2) Stomatal frequency for lowest stem-leaves, lower surface. (3) Thickness of palisade for lowest stem-leaves, measured in terms of total chlorenchyma thickness.

For the following structural features either the water supply was clearly without influence or else no consistent relation to water supply was apparent. (1) Stelar radius of root, both absolute and relative to root radius. (2) Number of xylem strands in root. (3) Laminar area of upper stem-leaves. (4) Stomatal frequency for root-leaves, lower surface and upper surface, for

lowest stem-leaves, upper surface, and for upper stem-leaves, upper surface. (5) Thickness of chlorenchyma for lowest stem-leaves and for upper stem-leaves. (6) Number of palisade layers for root-leaves, lowest stem-leaves and upper stem-leaves. (7) Thickness of palisade, absolute, for root-leaves, lowest stem-leaves, and upper stem-leaves. (8) Thickness of palisade, when measured in terms of chlorenchyma thickness, for root-leaves and upper stem-leaves.

The minimum water supply survived by the plants was accompanied by a postponement or total lack of stem elongation and flower production.

Soil-flooding, with submergence of the aerial portion of the plant, was accompanied either by early death or by an excessive elongation of the stem and a postponement of flower production, both of which made comparison difficult in regard to other features, especially on account of seasonal differences.

II. A second generation of *Ranunculus sceleratus*, with the environmental conditions aside from water supply considerably different from those of the first generation (especially in regard to light), and with different degrees of water supply similar to those employed in the first generation, gave rather different results from those just presented for the first generation, as to the relation of structural features to water supply. The following two features showed a consistent *direct* relation to water supply in *both* generations. (1) Thickness of stem cortex. (2) Thickness of stem aerenchyma, both absolute and relative to cortex thickness.

These two features are indicated by asterisks in the list of features directly related to water supply for the first generation (see p. 210). The other features studied for both first and second generations failed to show a direct relation to water supply in the second generation. In one case (number of achenes in tenth flower to open) the second generation showed an *inverse* relation to water supply; progressively *lower* water supply gave progressively *larger* values. It is thus evident (as might be expected from the nature of conditional control in general) that the effects of differences in water supply depend to a very marked extent upon just what is the complex of other environmental factors.

If we assume that the three different degrees of water supply (represented by the amphibious, mesophyte and subxerophyte groups), regarding which the two generations of *R. sceleratus* may be compared, were each practically the same for the second as for the first generation, the structural differences between the two generations, for each of the three degrees of water supply, may be studied. These differences may be regarded as related to the non-water-supply conditions of the two generations. With the high water supply of the amphibious group, the non-water-supply conditions of the second generation gave much smaller values than did those of the first, for all plant features involved in this comparison excepting three. This principle holds for the following structural features. (1) Total height. (2) Basal stem

diameter. (3) Thickness of stem cortex. (4) Thickness of stem aerenchyma, absolute. (5) Number of achenes per flower (for both the first and tenth flower to open). (6) Seed viability.

In regard to only one feature (thickness of stem aerenchyma in terms of cortex thickness) was the amphibious value for the second generation significantly *larger* than that for the first.

With the medium water supply of the mesophyte group the main general statement just made for the high water supply of the amphibious group also holds, with the same three exceptions. (Stem height to first flower was here alike for the two generations,—instead of being negligibly smaller in the second, as with the amphibious water supply; stem aerenchyma thickness in terms of cortex thickness was here greater for the second than for the first generations,—by about the same percentage as in the case of the amphibious group; and laminar area of root-leaves was here markedly greater (23 per cent.) in the second than in the first generation). With high and with medium conditions of water supply, therefore, the difference between the two environmental complexes aside from water supply, appears to have exerted the same general influence upon the plants of the two generations.

With the low water supply of the subxerophyte group, on the other hand, the influence of the non-water-supply complex was shown in a very different manner. For none of the plant features considered was the value markedly smaller in the second than in the first generation. All but three of these features gave markedly *larger* values for the second generation. This principle holds for the following features. (1) Total stem height. (2) Stem height to first flower. (3) Thickness of aerenchyma (both absolute and relative to cortex thickness). (4) Laminar area of root-leaves. (5) Number of achenes per flower, tenth flower. (6) Seed viability. In this case the three exceptions were basal stem diameter, stem cortex thickness and number of achenes in the first flower, for all of which an insignificant *decrease* is shown as from the first to the second generation. It will be noted that the exceptions here mentioned are not at all the same features as those that appeared as exceptions to the general rule for high and medium degrees of water supply.

For all these features by which the two generations may be compared, excepting stem cortex thickness, stem aerenchyma thickness (both absolute and relative to cortex thickness) and seed viability, the degree of water supply appears to have been effective in a perfectly consistent manner, to determine the influence exerted upon the plants by the change in non-water-supply conditions (from the first generation to the second). Excepting the features just mentioned, medium water supply gave a less pronounced decrease or a more pronounced increase, as from the first to the second generation, than did high water supply. In all cases, without exception, low water supply gave a less pronounced decrease or a more pronounced increase than did medium water supply.

III. A third generation of plants of *Ranunculus sceleratus* was grown with about the same treatment as had been given in the second generation, progeny of plants of the amphibious group of the second generation and of plants of the xerophyte group of the first generation being employed. These two progenies of very different forms gave practically the same values for laminar area of root-leaves, both with flooded and with very dry soil.

#### RANUNCULUS ABORTIVUS

The experiments with *R. abortivus*, carried out with different degrees of water supply, as in the first generation of *R. sceleratus*, but all with the same complex of environmental conditions aside from water supply (this complex being much the same as that for the first generation of *R. sceleratus*), showed a direct and consistent relation to water supply for certain structural features; for these features, progressively lower water supply gave progressively smaller values. The features are:—(1) Stelar radius of root, both absolute and relative to root diameter. (2) Number of bundles in root-leaf petioles. (3) Petiolar width, root-leaves. This same direct relation was inconsistently or partially indicated for the following additional features, however:—(1) Thickness of stem cortex. (2) Thickness of stem aerenchyma, both absolute and relative to cortex thickness. (3) Stem bundle interval, absolute. (4) Laminar area of root-leaves. (5) Thickness of root-leaf palisade, both absolute and relative to chlorenchyma thickness.

A consistent, though not very pronounced, *inverse* relation to water supply was shown by a single feature, stem bundle interval relative to cortex thickness. To this may be added the following features, for which an inverse relation was inconsistently or partially indicated:—(1) Thickness of root aerenchyma, both absolute and relative to root diameter. (2) Stomatal frequency of root-leaves, lower surface. (3) Number of root-leaf palisade layers.

No apparent relation to water supply was shown by the following features:—(1) Root radius. (2) Number of xylem strands in root. (3) Stomatal frequency of root-leaves, upper surface. (4) Number of layers of root-leaf chlorenchyma cells. (5) Thickness of root-leaf chlorenchyma.

The minimum water supply with which the plants survived was accompanied by a total lack of flower production and by maximum values for stomatal frequency and number of palisade layers of the root-leaves.

An increase of water supply beyond soil flooding, resulting in submergence of the aerial portion of the plant, was accompanied by early death in all cases.

#### COMPARISON OF *R. SCELERATUS* AND *R. ABORTIVUS*

Both species here studied agree, in regard to their relation to water supply, in several ways. The following features are those for which a direct relation (progressively smaller values with progressively lower water supply) is at

least suggested in both species. (1) Thickness of stem cortex. (2) Thickness of stem aerenchyma, both absolute and relative to cortex thickness. (3) Stem bundle interval, absolute. (4) Petiolar width of root-leaves. (5) Laminar area of root-leaves. A consistent inverse relation (progressively larger values with progressively lower water supply) is consistently shown for both species in regard to stem bundle interval relative to cortex thickness.

The two species both fail to show any apparent relation to water supply in regard to two features:—(1) Number of xylem strands in root. (2) Stomatal frequency, upper surface of root-leaves. They disagree in their relation to water supply for the following features:—(1) Root radius. (2) Stellar radius of root, both absolute and relative to root radius. (3) Thickness of root aerenchyma, both absolute and relative to root radius. (4) Stomatal frequency of lower surface of root-leaves. (5) Number of layers of chlorenchyma. (6) Thickness of root-leaf chlorenchyma. (7) Number of layers of root-leaf palisade. (8) Thickness of root-leaf palisade, both absolute and relative to chlorenchyma thickness.

## INTRODUCTION

### THE GENERAL PROBLEM

The response of plants to environmental conditions has been studied in various ways, the response and some of the conditions having been sometimes more or less definitely measured. Plants have been observed in the field and also with some of the conditions controlled. Various environmental factors have been singled out and the effects of differences in a given factor have been somewhat definitely determined in some cases. However, observations have usually been made on the effects produced upon only a single organ or upon one function of a plant,<sup>2</sup> so that the relation of general development to environment has not yet received much serious attention. The aim of the work reported in the present paper has been to determine quantitatively the responses produced in the development of two plastic species, with regard to certain differences in the surroundings, especially in soil-moisture content, and to determine as far as possible any correlations that may be manifest between structural differences occurring in various parts of the plant, on the one hand, and the environmental conditions, on the other.

It seems that both the ecology of wild plants and scientific agriculture are to be advanced by the sort of study here attempted.<sup>3</sup> In order to really

<sup>2</sup> E. g., Briggs, L. J., and H. L. Shantz, The water requirement of plants. II. A review of the literature. U. S. Dept. Agric., Bur. Plant. Ind., Bull. 285. 1913.

<sup>3</sup> This is shown by similar studies which are as follows: Clements, Edith S., The relation of leaf structure to physical factors. Trans. Amer. Microsc. Soc. 1905: 19-102. 1905. (This paper contains a summary of much previous work. No conclusions could be drawn concerning the effect, upon polydemic species, of a difference in the water supply alone, because any such difference was practically always accompanied by a difference in humidity or light, or both.) Kiesselbach, T. A., Transpiration as a factor in crop production. Nebraska Agric. Exper. Sta. Research Bull. 6. 1916. (This paper gives an extensive bibliography on certain relations of plants to their water supply.) Yunker, T. G., A study of the relation of soil moisture to transpiration and photosynthesis in the corn plant. Plant World 19: 151-161. 1916.

understand any natural process or phenomenon, it is first necessary to have quantitative knowledge of the manner in which the controlling conditions operate to produce the observed effects. It is necessary to have quantitative knowledge of the relations holding between the internal conditions that directly determine the various growth processes, etc., of plants, and the influential conditions of the surroundings. It of course follows that the artificial control of phenomena such as those of plant growth, as in agriculture, can not be very definite without this same sort of quantitative understanding regarding the responses of cultivated plants to environmental conditions. Of course many kinds of plants require study, for different species differ internally and behave differently with regard to the same set of environmental conditions, but each particular contribution must deal with a small number of forms. In the earlier stages of this kind of work, it seems desirable to employ what are called "plastic" species, that is, plants that are known to exhibit rather pronounced responses to environmental differences; the necessary measurements and comparisons are thus more readily made.<sup>4</sup> The two species of *Ranunculus* dealt with in the present paper (*R. sceleratus* L. and *R. abortivus* L.) are examples of such plastic forms, which is the reason why they were selected. While the methods here employed are realized as being capable of marked improvement, and while the results obtained are not as definite as future studies will surely bring to light, it is hoped that the outcome of this investigation may serve to advance, to some degree, our knowledge of the relations of plants to their surroundings.

This investigation was suggested by Doctor Frederic E. Clements, under whose direction it has been carried on. Grateful acknowledgment is here made for many helpful suggestions from Dr. Clements, and for the facilities placed at the writer's disposal by the Department of Botany of the University of Minnesota. Many suggestions as to the manner of presentation of this paper have been made by the Managing Editor of *Physiological Researches*.

#### THE PLANTS EMPLOYED

In a discussion of the application of culture methods to the possible origin of new forms by response to variations in the water content of the soil, F. E. Clements<sup>5</sup> described experiments with *Ranunculus sceleratus* L., in which "nine new forms . . . showed striking differences in vigor and growth . . . accompanied by distinct and often striking differences in the number and position of the stomata, the amount of sponge and palisade

---

<sup>4</sup> This is seen in the following: McCallum, W. B., On the nature of the stimulus causing the change of form and structure in *Proserpinaca palustris*, Bot. Gaz. 34: 93-103. 1902. Burns, G. P., Heterophylly in *Proserpinaca palustris* L. Ann. Bot. 18: 579-587. 1904. MacDougal, D. T., The determinative action of environic factors upon *Neobeckia aquatica* Greene. Flora 106: 264-280. 1914.

<sup>5</sup> Clements, F. E., Research methods in ecology. xvii + 334 p. Lincoln, 1905. See p. 158.

tissues, and the development of air passages." These preliminary results, indicative of much plasticity and tolerance, pointed to this plant as being well suited for the present investigation. The desirability of using this species was increased still more by its having many simple pistils, its suppression of all the basal buds except the terminal one, its rather small size, the large number of root-leaves and their uniform shape, its determinate inflorescence, the ease of hand-pollination, and its short life cycle. All of these characteristics facilitate comparative measurement and the comparison of different plants in regard to any particular characteristic. Another species, *Ranunculus abortivus* L. was also used, although it did not appear, or finally prove, to be as satisfactory.

As it grows in the open in Nebraska, *Ranunculus sceleratus* attains the rank of a secondary species in the meadow formation of the rush-sedge—wet-meadow association, as is pointed out by Pool.<sup>6</sup> Its various European forms have been described by Glück.<sup>7</sup> Six forms are considered by him; normal land, dwarf, marsh, floating-leaf, shallow-water, and submerged.

## METHODS OF EXPERIMENTATION

*Soils used.* The soil was uniform for all the individuals of each set of plants. For the first generation, for both species, a "garden soil" was used. This was a medium sand, according to the mechanical analysis.<sup>8</sup> It was black with humus and, with the Hilgard<sup>9</sup> 1-cm. column, displayed a water-retaining capacity of 46.7 cc. per 100 cc. of soil (113.1 g. of dry soil). As the soil was placed in the pots, any large masses of humus occurring in it were removed.

For other sets of plants, grown subsequently to those just mentioned, a soil was employed that consisted of a mixture of equal volumes of this garden soil and a humus-free white sand. The latter was obtained from a layer of sandstone exposed by erosion in the Mississippi gorge within the city of Minneapolis. Before mixing, both the soil and the sand were air-dried and passed through a sieve with openings 5 mm. square. The mixing was done by hand, each potful being prepared separately. Since the sand was practically free of humus the percentage variation in humus content in the mixture must have been reduced by half, while the vigor of the plants grown in the mixture was not noticeably different from that exhibited by plants in the garden soil. The mixture thus produced was also a medium sand, according

<sup>6</sup> Pool, R. J., A study of the vegetation of the sandhills of Nebraska. *Minnesota Bot. Studies* 4: 189-312. 1931. See p. 292.

<sup>7</sup> Glück, H., Biologische und morphologische Untersuchungen über Wasser- und Sumpfgewächse. 3 vols. Jena, 1905-1911. See p. 508 of vol. 3.

<sup>8</sup> Jackson, J. L., and E. O. Tappin, The principles of soil management. xxviii + 531 p. New York, 1909. See p. 77.

<sup>9</sup> Hilgard, E. W., Soils, their formation, properties, composition, and relations to climate and plant growth in the humid and arid regions. xxx + 593 p. New York and London, 1910. See p. 209.

to the mechanical analysis, and with the Hilgard 1-cm. column it displayed a water-retaining capacity of 40.4 cc. per 100 cc. of soil (139.1 g. of dry soil).

*Potting.* The method of potting was uniform for all the individuals of any generation of plants. About 900 cc. of soil was used for each plant, in a 12.5-cm. clay pot. A thin pad of dead sphagnum moss was put in the bottom of each pot to facilitate the entrance of air or of water and to hold the soil in the pot. The soil used for the first generation, in each case, was packed by hand while slightly moist; for subsequent cultures it was poured into the pot while air-dry and was thus always packed automatically and with considerable uniformity.

*Control of soil-moisture content.* The submerged-leaf and floating-leaf cultures were in kegs. For the amphibious plants the pots stood in jars of water, so that the soil and parts of the petioles of the lower leaves were always submerged. For the mesophytes the pots stood in saucers of water, so that the lower part (about 30 cc.) of the soil mass was flooded and the upper portion was always plentifully supplied with water drawn up from below.

The subxerophyte cultures were supplied with water either by hand and at intervals or else automatically by auto-irrigators. For the first generations, water was added daily at the soil surface, in a depression close to the plant, where it was quickly absorbed. This method proved unsatisfactory, for several reasons. A daily periodicity in soil-moisture content resulted from the fact that water was added only once a day. Weather variations, with fluctuating evaporation and transpiration rates, produced irregular variations in the soil-moisture condition. Finally, a seasonal decrease in soil-moisture content accompanied the progressive increase in the amount of water required by the growing plant. Partially successful attempts were made to reduce these variations by regulating the daily additions of water according to the moisture content of the soil and according to the amount which was needed daily by the mesophyte cultures, but auto-irrigation<sup>10</sup> was resorted to for the later cultures. A 13-cm. porous clay cylinder (such as is supplied by the Plant World), properly connected with a water reservoir below, was held in place within the pot as the air-dry soil was poured in, after which the pot was set in a saucer of water until the soil became thoroughly wet, and was then removed from the saucer. The surplus of water thus placed in the soil disappeared in a few days. The desired soil-moisture content was secured by adjusting the level of the reservoir as related to that of the porous cylinder.

The xerophyte cultures were the driest of the series and were furnished with little more water than what was necessary for life. Any occasional delay

<sup>10</sup> Livingston, B. E., A method for controlling plant moisture. *Plant World* 11: 39-40. 1908. Hawkins, L. A., The porous clay cup for the automatic watering of plants. *Plant World* 13: 220-227. 1910. Livingston, B. E., and L. A. Hawkins, The water-relation between plant and soil. *Carnegie Inst. Wash. Pub.* 204: 1-48. 1915. Holmes, F. S., Moisture equilibrium in pots of soil equipped with auto-irrigators. *Johns Hopkins Univ. Circ.* March, 1917, p. 208-210.

in the regular daily addition of water usually resulted in wilting, and other plants that were furnished with less water soon died. The small amount of water supplied to each plant was not enough to wet the soil mass equally throughout, but the soil in the center of the pot and near the suppressed stem was always moist enough to keep the plant alive. Automatic watering did not prove to be practicable for this class of cultures, apparently because the control had to be maintained by keeping part of the soil below its capillary wetting point.

The same water was used for all plants, drawn from the Minneapolis water system. The roots of the amphibious plants and the mesophytes grew through the hole in the bottom of the pot, into the water below, and the projecting portions were removed at intervals of a week or less, to keep the volume occupied by the root systems more nearly the same in all cases. The pots used for the mesophytes, the subxerophytes, and the xerophytes, and the saucers used for the first group, were water-proofed with orange shellac. This was applied as a saturated alcoholic solution when the porous clay was dry and was finally baked on, at  $104^{\circ}\text{C}.$ , for two days. The extent to which the soil-moisture content was controlled was shown by determinations made as described below.

*Determination of soil-moisture content.* For the mesophytes, subxerophytes, and xerophytes, a core of soil 2.3 cm. in diameter and 10 cm. long was taken with a cork borer midway between the plant and the wall of the pot. This core was used as a soil sample for determining the moisture content of the soil, drying being accomplished at  $104^{\circ}\text{C}.$  For the amphibious plants the soil sample was removed with a spoon. In each pot successive samples for different determinations were taken in definite order from different regions of the pot—proximal, left, right, and distal—so that the same region was not sampled twice. This rule was not observed exactly when the porous cylinder was present, since it was in the proximal region of the pot. Each core was returned, or was replaced with similar soil, the soil being moistened when placed in the opening. The data derived from these soil-moisture determinations will be given below with the other data on the various cultures, being expressed as soil-moisture contents in terms of percentage of the dry weight of the soil.

*Light, temperature, and air humidity.* The natural tendency for the conditions of light, air temperature, air humidity, and soil temperature, to alter for the different plants was considerably reduced by the greenhouse environment, by a cloth netting which covered all the cultures (to exclude bees, etc.), and by the arrangement of the plants. The netting, with meshes about  $0.9 \times 1.4$  mm. and with threads about 0.1 mm. thick, enclosed a space  $2.5 \times 0.75 \times 0.75$  m. above and on the sides. In this space the cultures were distributed so that each occupied the center of an area  $19.5 \times 19.0$  cm., and so that there were four rows consisting, successively, of amphibious, xerophytic,

subxerophytic, and mesophytic cultures. The xerophytes and subxerophytes stood between the amphibious plants and the mesophytes, in order to give all plants about the same air humidity. The light for all cultures was natural sunlight modified in quality and intensity in several ways. Direct and indirect sunlight first passed through the roof of the greenhouse (which was not clean) in all cases. It also passed through a water layer for the submerged-leaf plants and through the cloth netting, mentioned above, for the others. During the summer of 1916, the greenhouse was shaded by the usual coating of white paint applied to the glass. There was no shading of one plant by another. Some comparisons of the photographic light intensities were made in August by means of the Clements [1905] photometer, which employs photographic paper. According to these measurements, direct noon sunlight was reduced by the unpainted glass to 22.5 per cent. of its intensity in the open, an intensity almost as low as that of natural noon light on a gloomy, rainy day in the open. Direct noon sunlight was reduced still more, to 3.5 per cent., by the painted glass of 1916. The netting showed but little effect in modifying the photometer readings. In general, it may be said that while all these plants were subjected to light variations corresponding to the natural fluctuations due to weather, time of day and season, all plants not under water received sensibly the same kinds and intensities of light, and all the submerged cultures were subjected to like light conditions, although those for the latter group were of course more or less markedly different from those for the former one.

Simultaneous determinations of air humidity and of temperature were made on several types of days. The former were made with a cog psychrometer such as that described by Clements [1905] and the latter with several standardized thermometers readable to  $0.1^{\circ}\text{C}$ . Temperature readings were taken in the air 15 cm. above the soil, at the surface of the soil, and in the soil at a depth of 5 cm. These determinations indicate that, while air temperature, soil temperature, and air humidity varied considerably from time to time, each of these three conditions was about alike for all but the submerged plants. Of course the plants of the latter group were not subject to air conditions at all, while the temperature of the water surrounding these plants followed (by conduction and radiation of heat) the general air temperature. The outdoor air temperatures for four representative days, March 8, 9, 13, and August 8, 1916, were, respectively,  $-16^{\circ}$ ,  $5^{\circ}$ ,  $-1^{\circ}$ , and  $27^{\circ}\text{C}$ . During winter the use of steam heat within the greenhouse practically annulled the influence of outdoor temperature upon the plants. The temperature and humidity values obtained at about noon on the dates just mentioned are given in table I. Cloudiness on March 13 made the indoor temperature lower than on the other two March days, which had low outdoor temperatures but were sunny. When the ventilator in the roof-peak of the greenhouse was open there were some sensible differences between different regions

of the air outside of the netting enclosure, but none were manifest within the enclosure. Thus on March 9 a cold draft from the open ventilator caused the air south of the enclosure to be more humid than the air over the keg of water, for which a determination had been made just previously.

*Animals, fungi and algae.* The netting served to exclude from the enclosure insects that might carry pollen, other than thrips.<sup>11</sup> The fumigation carried out by the gardener did not eliminate white flies or thrips, but the former were entirely eradicated and the latter kept to small numbers by fortnightly brushing of the affected plant parts with a diluted commercial nicotine solution. Air-drying and sifting the soil removed the larger animals, such as angle worms. Powdered sulphur was employed to check occasional epidemics of powdery mildew. Various algae appeared on the water and plant surfaces in the cultures of the submerged-leaf group, and these were occasionally removed.

TABLE I

*Temperature and relative humidity, on four representative days of 1916, for the various cultures. Temperature readings were taken in the air 15 cm. above the soil, at the surface of the soil, and in the soil at a depth of 5 cm.*

CULTURE	TEMPERATURE, DEGREES C.												AIR HUMIDITY (RELATIVE), PER CENT.			
	March 8			March 9			March 13			August 8						
	Air		Soil	Air		Soil	Air		Soil	Air		Soil				
	Above soil	At soil surface		Above soil	At soil surface		Above soil	At soil surface		Above soil	At soil surface					
	Above soil	At soil surface		Above soil	At soil surface		Above soil	At soil surface		Above soil	At soil surface					
Above soil	At soil surface	Above soil		At soil surface	Above soil		At soil surface	Above soil		At soil surface						
Amphibious.....	25.6	22.0	20.8	27.2	27.4	27.0	16.2	14.6	13.6	30.7	27.4	24.2	60	50	74	49
Mesophyte.....	26.2	22.0	21.0	30.0	26.8	26.5	16.6	14.4	13.9	30.4	27.3	25.6	57	42	74	43
Subxerophyte.....	26.2	21.9	20.9	28.8	26.5	26.2	16.2	14.4	14.2	31.5	28.5	25.9	57	50	74	44
Xerophyte.....	26.4	21.9	20.8	28.7	28.4	28.2	16.3	14.5	14.7	31.3	29.1	26.6	60	41	74	43

## EXPERIMENTS AND RESULTS WITH RANUNCULUS SCCELERATUS, FIRST GENERATION (1915)

### GENERAL CONSIDERATIONS

All of the seeds used in 1915 came from a single thrifty plant, a volunteer growing in a tub of wet soil in the greenhouse. These seeds were sown on moist soil in May, 1915, and the seedlings were potted separately in early July.

The purely vegetative period, terminating in the first opening of flowers, extended, for each plant of the amphibious, mesophyte, and subxerophyte

<sup>11</sup> Shaw, H. B., Thrips as pollinators of beet flowers. U. S. Dept. Agric. Bull. 104. 1914. Castle, W. E., Selection, sugar-beets and thrips. Amer. Nat. 49: 121-122. 1915.

groups, to a date between about August 20 and September 25. The full duration of the period of flowering was not determined; a rapid increase in the number of thrips on the flowering stems, together with the great difficulty of removing such insects from this part of the plant, led to the discontinuing of the cultures as soon as a supply of ripe fruits was secured. Forty-two days elapsed after September 25 before the occurrence of any flowering in the xerophyte group, and 222 days before any in the submerged-leaf group. Most of the xerophyte plants produced no flowers, but continued their vegetative growth without any marked changes until removed for examination. Seven of the eight submerged-leaf plants died during the fall and winter. The survivor showed an interesting combination of two vegetative periods. At first, as is usual for the species when growing in soil and air, a number of crowded root-leaves were produced and then the stem elongated upward from the center of the rosette thus formed. But, instead of this stem producing progressively smaller leaves and finally flowers (as is the common occurrence), it produced adventitious roots and progressively *larger* leaves. When the tip had reached the water surface, about 40 cm. above the basal roots, a second rosette of leaves was produced, each of which was in the air excepting for the lower end of the petiole, which was submerged. From the second rosette came a normal flower-bearing stem, so that the keg contained a plant apparently like those of the amphibious group. In fact, the early decay of the submerged portion of the stem made the transition of this individual from the submerged-leaf condition to the amphibious condition real and complete, except for its lack of direct contact between roots and soil particles.

The details of the life histories of the various plants of the generation of 1915 are shown graphically by the diagram of figure 1. The letters at the left denote the environment groups, A, M, SX, X, and S referring respectively to the amphibious, mesophyte, subxerophyte, xerophyte, and submerged-leaf groups. The numbers at the left stand for the individual plants in the various groups, similar numbers indicating, except for the submerged-leaf plant, similar positions in the four parallel rows of cultures (see p. 218). The lengths of the horizontal bands denote the growth periods from seed. The leftward-pointing arrows denote that the exact time of seed planting was not recorded in these cases, the arrows ending at the right and joining the bands for the date at which the soil-moisture content, etc., was brought under control. The checkered bands represent the lengths of known periods from seed-sowing to the establishment of control conditions. Oblique lines denote that the exact date of the first flower was not recorded; with this exception, the solid portion of each band represents the portion of the culture period subsequent to the opening of the first flower. Dates, from June 19, 1915, to March 25, 1916, are indicated near the upper margin of the diagram, and later ones (for the continued portion of the band for the single submerged-leaf culture, S108), till July 4, 1916, are shown near the lower margin.

The data obtained on the soil-moisture conditions, for the four groups of cultures in which the leaves were not submerged, are shown graphically in the diagram of figure 2, where group letters and culture numbers appear at the left and the soil-moisture percentages (on the basis of dry weight) are noted at the top. Dates of sampling are indicated at the right of the horizontal bands, each of which shows by its length the percentage of moisture found at that time, in that particular pot. Unshaded bands indicate moisture-contents of soil samples, each of which was obtained just after water had been added to the pot; checkered bands represent moisture-contents of

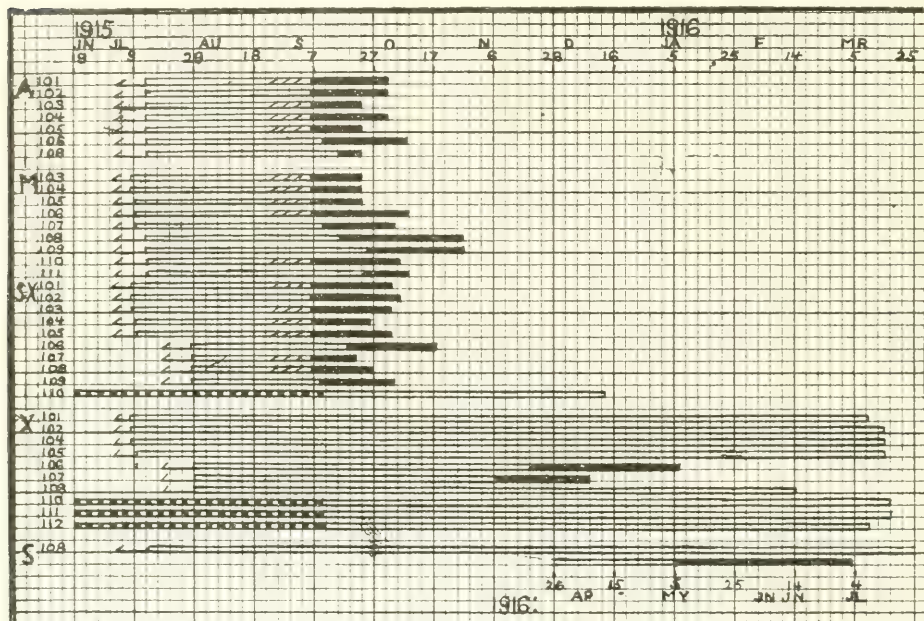


FIG. 1. Diagram showing details of the life-histories of the five groups of plants of the first generation (1915) of *Ranunculus sceleratus*. For description see text, p. 221.

samples obtained just before water was added; other samples are represented by solid bands. The averages of the several data, for each single date for each group of cultures, are shown in the form of graphs in figure 3. These graphs represent (for each environment, or culture group) the march of the soil-moisture content as it fluctuated from time to time, in so far as data for this were obtained. The dates for the graphs are indicated above. The percentage values are indicated at the left.

A study of the data shown in figures 1 and 2 brings out the fact that, in any single group, the individual differences in the time of the first flowering

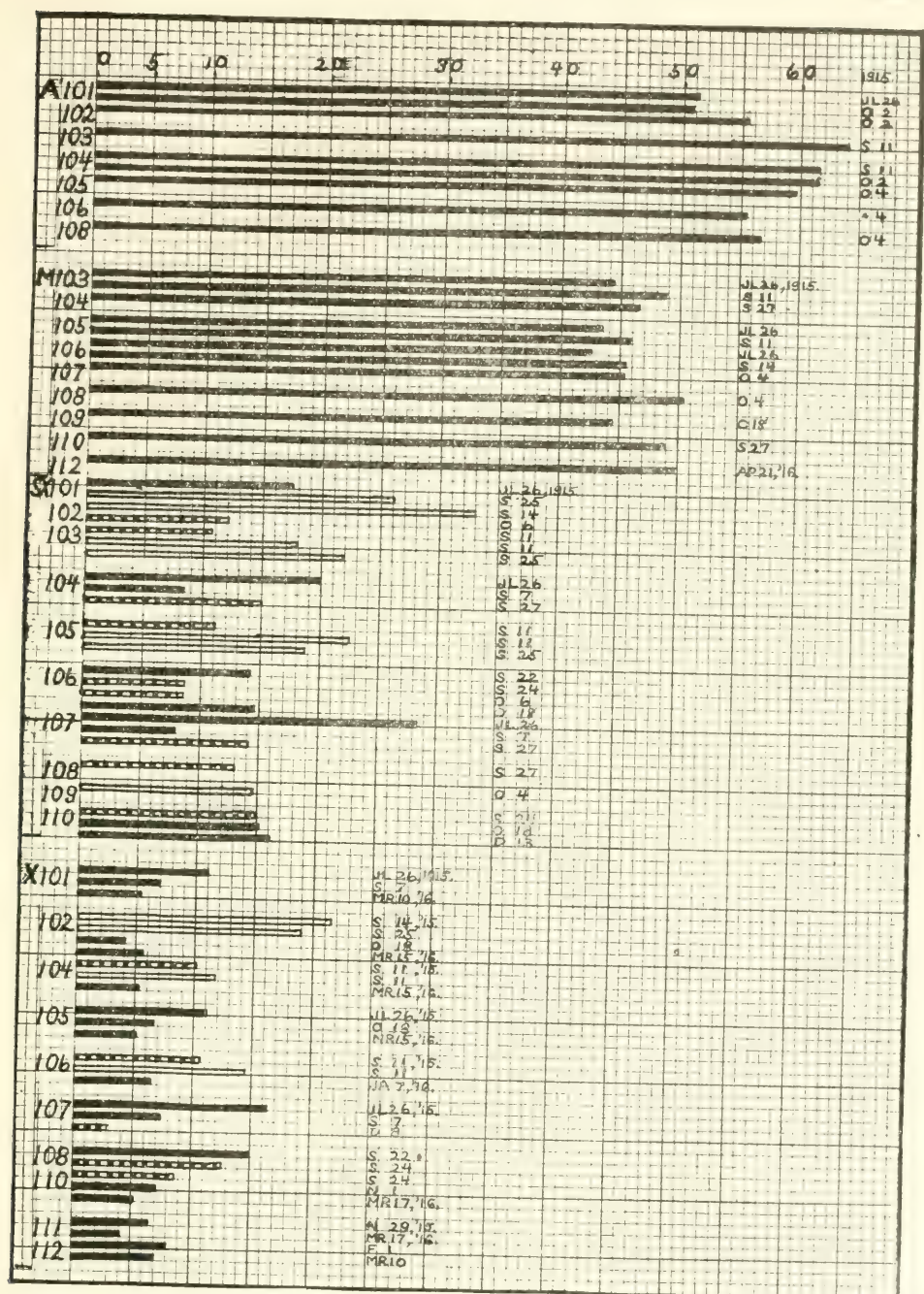


FIG. 2. Diagram showing soil-moisture contents of the various pots at several dates during the culture period of the first generation (1915) of plants of *Ranunculus sceleratus*. For description see text, p. 222. Compare with figure 3.

did not correspond to the individual differences in soil-moisture content. The soil-moisture content for the submerged-leaf plants may be taken to be the same as that for the amphibious ones, varying from 50.8 to 64.2 per cent. The soil-moisture content for the mesophytes, subxerophytes, and xerophytes, respectively, varied between 42.6 and 50.5, between 7.9 and 28.4 and be-

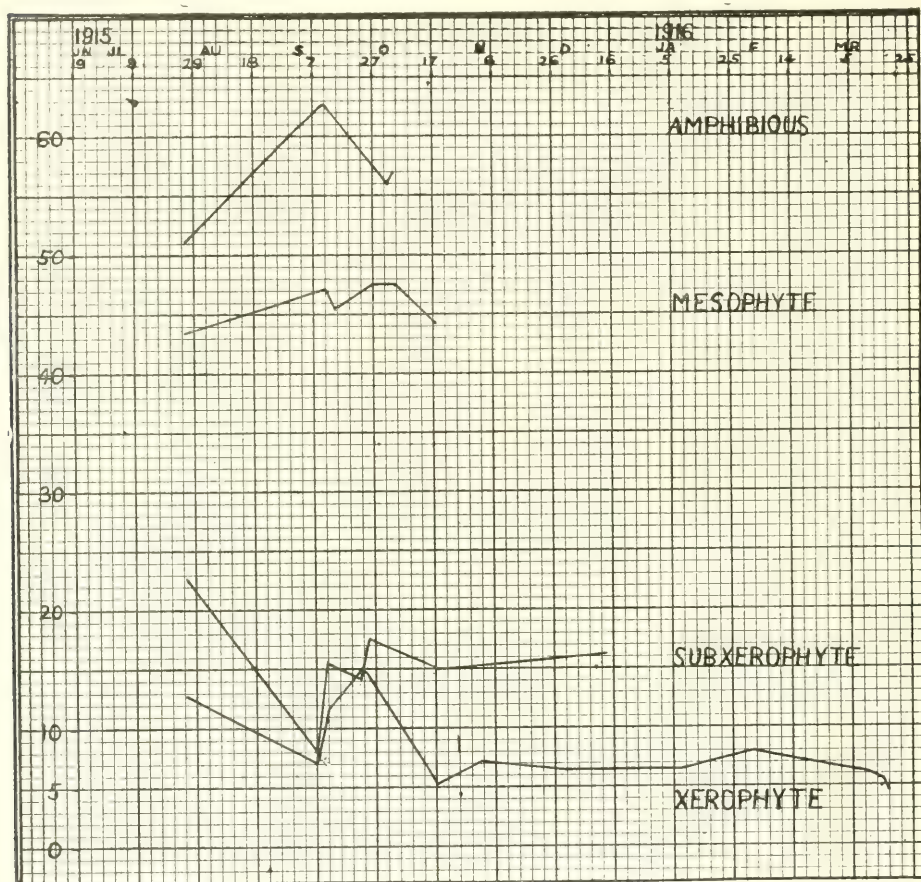


FIG. 3. Graphs showing the march of the average of soil-moisture content for each group of plants (*Ranunculus sceleratus*, first generation), as obtained by soil samples taken from the pots from time to time. For description see text, p. 222. Compare with figure 2.

tween 4.2 and 16.4 per cent. The overlapping of the last two ranges may be considered as of little importance, because of its being restricted to the period before October 17 (see figs. 2 and 3) and because all the plant structures from which the data for the xerophytes were obtained were formed after October 17, when this range was from 4.2 to 8.1 per cent. The means of the

extremes of these ranges are 57.5, 46.6, 18.2 and 6.2 per cent. When these means are compared with the first, that of flooded soil, taken as the standard, they are found to have the relations, 100:81:32:11.

## ROOTS

The root material was killed in acetic-alcohol mixture<sup>12</sup> and preserved in 70-per cent. alcohol. To obtain representative sections, each root selected for sectioning was of about average age, size, and position on the stem, and paraffin sections were prepared from the various roots at about the same distance from their junction with the stem. By means of the prepared sections a comparative study was made of four anatomical features: root radius,

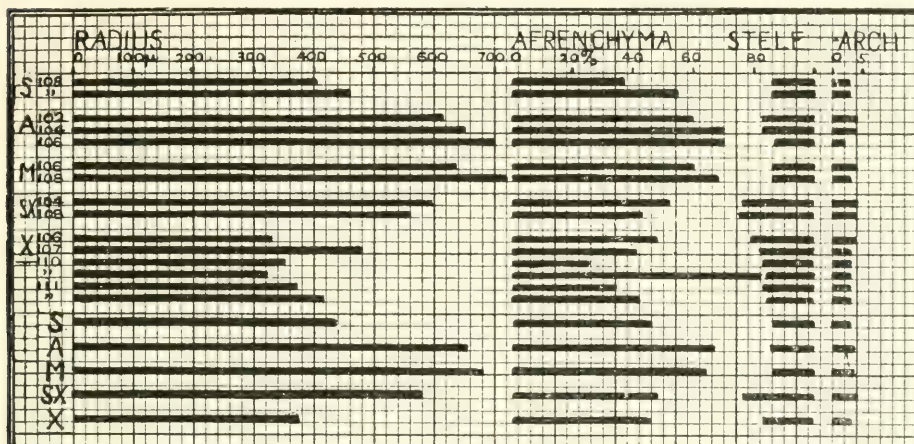


FIG. 4. Diagram showing numerical data for roots of the first generation (1915) of plants of *Ranunculus sceleratus*, the plants grown with five different environmental complexes. For description see text, p. 226.

thickness of aerenchyma (both absolute and as a percentage of the root radius) stelar radius (both absolute and as a percentage of the root radius) and number of xylem strands in the stele. That portion of the subepidermal region of the root in which the intercellular spaces appeared in the cross section as larger than the cells themselves was considered as aerenchyma. The thickness of this layer, and also the radius of the stele, were first measured in micra, and the data were afterwards reduced to terms of percentage of the root radius. In cases where the thickness of the aerenchyma was different in different radii of the section, it was measured in several radii and the average of the several results was used as the thickness value.

<sup>12</sup> Chamberlain, C. J., Methods in plant histology. xi + 314 p. Chicago, 1915. See p. 19.

The data obtained from these measurements are shown diagrammatically in figure 4, the lengths of the horizontal bands representing the data in question. The column of bands for number of strands of xylem is designated by the sign “-arch.” The scale of units for each column of bands is shown at the top; the root radius is in terms of micra and the thickness of aerenchyma and the radius of the stele are in terms of percentage of the root radius. It will be noted that the same scale of units serves for both aerenchyma and stele. The upper portion of the diagram represents the actual data obtained, each band being designated, at the left, by letter and number denoting environment group and individual plant, with the scheme of notation the same as in the diagrams of figures 1 and 2. The lower part of the diagram of figure 4 (five bands) represents the averages for the various groups. The averages just mentioned, together with their probable errors,<sup>13</sup> are presented in table II,

TABLE II

*Average numerical data for roots of Ranunculus sceleratus of the first generation (1915), the plants grown with five different environmental complexes*

ANATOMICAL FEATURE		SUB-MERGED GROUP	AMPHIBIOUS GROUP	MESO-PHYTE GROUP	SUBXERO-PHYTE GROUP	XEROPHYTE GROUP
Root radius, micra .....		435	655±18	<b>678±36</b>	578±15	375±17
Stelar radius of root	Absolute, micra.....	58	99±5	95±6	<b>140±0</b>	66±3
	Relative, per cent. of root radius...	14	15±1	14±.2	<b>24±.6</b>	17±.5
Number of xylem strands in root .....		3.0	3.3±.5	3.5±.4	<b>4.0±0</b>	3.2±.1
Thickness of root aerenchyma	Absolute, micra.....	195	<b>438±28</b>	<b>438±45</b>	273±31	170±5
	Relative, per cent. of root radius..	45	<b>67±3</b>	65±3	47±4	45±5

which is self-explanatory. Each of the averages, except for the single submerged-leaf plant, is derived from two or more values obtained from different plants and individually represented in the upper part of the diagram of figure 4. The interpretation of the probable error values, given after the sign ± in each case, will be considered when pointing out generalizations; if the difference between two values is covered by the sum of the associated error values, the difference is not regarded as being significant. In table II the maximum value for each feature is indicated by full-face type, the minimum by *Italics*.

<sup>13</sup> Zeleny, A., and H. A. Erikson, A manual of physical measurements. vii + xv + 147 p. New York, 1912. See p. 214. Briggs, L. J., and H. L. Shantz, The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agric., Bur. Plant Ind., Bull. 230. 1912. See p. 15. *Idem.*, The water requirement of plants. I. Investigations in the great plains in 1910 and 1911. U. S. Dept. Agric., Bur. Plant Ind., Bull. 284. 1913. See p. 14.

A study of the average values shown in table II brings out the following generalizations. The root radius was greatest for the mesophyte and smallest for the xerophyte group, being smaller both with reduced and with increased water supply, as compared with that of the mesophyte group. This difference of stele is like that pointed out by Bondonis,<sup>14</sup> who found that roots of mesophytic trees when submerged showed a smaller amount of supporting, conducting, and ligneous tissue, as well as an increase of lacunae.

The actual stelar radius (table II) proved to be greatest for the subxerophyte group, but did not appear to be generally related to the water supply; it was lowest for the submerged group and nearly as low for the xerophyte group. The relative stelar radius (per cent. of root radius) was also greatest in the subxerophyte group, and, although the order of magnitudes is not quite the same in both cases, the generalization from the average values for this feature is the same as for the absolute stelar radius. Evidently the stele varied in actual thickness, in this series of plants, almost proportionally to the total thickness of the root, except in the subxerophytes. The number of xylem strands in the root was greatest for the subxerophyte group, thus being like the actual and relative values for the stelar radius. The aerenchyma was thickest for the amphibious and mesophyte groups and thinner for the groups having smaller or greater water supplies. The relative thickness of aerenchyma (per cent. of root radius) leads to the same generalization as does the absolute thickness.

As is shown by the averages (S, A, etc., of figure 4, and table II) the amphibious and the mesophyte plants were foremost in regard to thickness of roots, and in regard to both absolute and relative thickness of the aerenchyma region, both submerging and reduction of water supply being accompanied by reduction in these features. Also, the subxerophyte plants were foremost in regard to both absolute and relative thickness of stele and number of xylem strands, while the minimum development of these features occurred in the submerged and xerophyte cultures.

#### STEMS

When the cultures were discontinued, the total height of the plant, the height to the first flower and two measurements of the stem diameter were determined. To get representative sections of the stems, these were always secured about 2 or 3 cm. above the rosette of root-leaves. The stem features studied from the prepared slides were thickness of cortex, thickness of aerenchyma (both absolute and relative to thickness of cortex) and distance between the fibro-vascular bundles (absolute, relative to thickness of cortex and relative to stem diameter).

Since the production of the first flower ended the elongation of the main stem and was followed by much forking in the flower-bearing part of the

---

<sup>14</sup> Bondonis, G., Contribution a l'étude de l'influence du milieu aquatique sur les racines des arbres. *Ann. Sci. Nat. Bot.* IX, 18: 1-24. 1913.

plant, both the total height of the plant and the height of the main stem to the first flower were measured from the level of the root-leaves. The two values for stem diameter employed in this study were a basal one and a median one. The former measurement was made in the region where the cross sections were to be obtained and the latter at a point half way between the leaf rosette and the top of the whole plant. A micrometer caliper, reading to 0.01 mm., was used. The thickness of the cortex was determined by measuring (on prepared cross sections) the radial distance from the epidermis to the cambium, or to the junction of phloem and xylem. As was true for the roots, the subepidermal region in which the intercellular spaces were larger than the cells themselves was considered as aerenchyma. The values were obtained just as for the roots. The average distance between the bundles was obtained by determining the average of the distances between the middle points of the cambium strips of succeeding bundles. These mean bundle intervals were afterwards expressed in two relative ways, as percentage of the corresponding cortex thickness and as percentage of the corresponding stem diameter. Each of the numerical values finally employed is the average of two or more data secured from different plants.

Most of the results of the various individual measurements and the group means are presented in the diagram of figure 5. The notation in this diagram is similar to that employed for the root data (fig. 4), the upper portion embracing data for individual plants and the lower the group means. Group letters and culture numbers occur at the left, and the scales of units appear above their respective columns of bands. Cortex thickness is denoted by the word "Cortex." The actual averages of bundle intervals are denoted by the sign "Bun. Dis.," and the relative ones (percentage of cortex thickness) are indicated by the sign "Bun. %." The abbreviation "Aer. %" signifies the data for thickness of aerenchyma expressed as percentage of cortex thickness. The stem height to the first flower is represented by the shaded portions of the height bands, the full lengths of these bands representing the total aerial height of the plants. Similarly, the shaded portion of each diameter band stands for the median diameter value and the entire band represents the basal diameter value, the latter corresponding to the stem region from which the cross sections were obtained. Each of the group means (lower portion of diagrams), except for the single submerged-leaf plant, is an average of the values given above in the diagram for the corresponding individual cultures.

The group averages and their probable errors are presented together in table III, which is arranged like table II, and is self-explanatory. The maximum of each group is indicated by full-face type, the minimum by italics.

The average or mean group values shown in table III and figure 5 may be interpreted to bring out the following generalizations. Both total height and height to the first flower were greatest for the amphibious group and

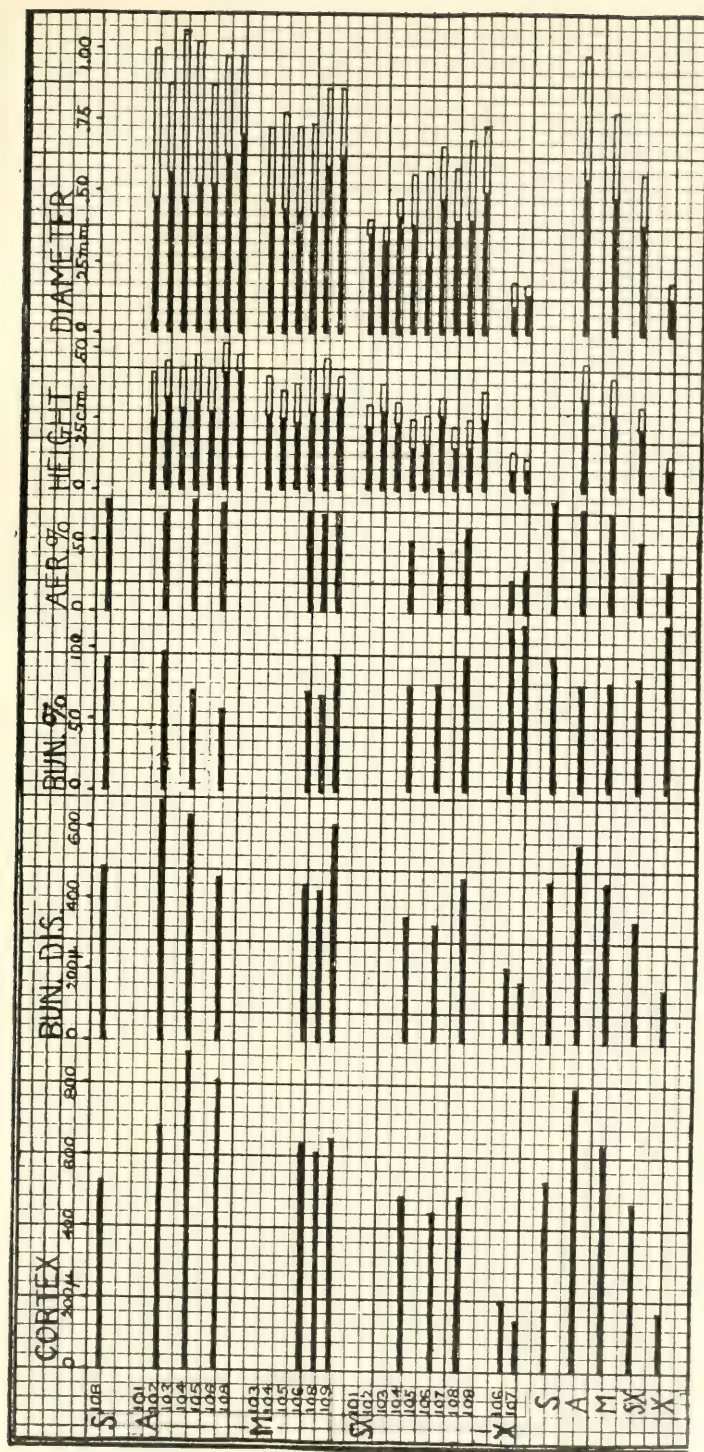


FIG. 5. Diagram showing numerical data for stems of the first generation (1915) of plants of *Ranunculus sceleratus*, the plants grown with five different environmental complexes. For description see text, p. 228.

progressively smaller for the other groups, in the order of their water supply, and the same relation holds for all the other anatomical features studied, excepting the two relative values for bundle interval. For each of these two features, the largest mean value is for the xerophyte group, and the values are smaller from group to group, in the inverse order of the amount of the water supply, except in regard to the submerged-leaf group. The differences in stem height are evident in figure 6, where representative cultures of the various groups are illustrated.

TABLE III

*Average numerical data for stems of Ranunculus sceleratus of the first generation (1915), the plants grown with five different environmental complexes*

ANATOMICAL FEATURE		SUB-MERGED GROUP	AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXERO-PHYTE GROUP	XEROPHYTE GROUP
Total height of plant, cm .....		—	45±1	39±1	29±1	13±1
Stem height, to first flower, cm .....		—	33±2	28±1	21±1	7±0
Basal diameter, mm .....		—	9.7±.2	7.9±.2	5.6±.3	1.8±.1
Median diameter, mm .....		—	6.6±.2	4.9±.2	3.9±.2	1.2±.2
Thickness of cortex, micra .....		530	790±50	630±10	470±10	160±20
Thickness of aerenchyma	Absolute, micra....	400	580±50	440±0	240±10	40±0
	Relative, per cent. } of cortex thick- ness	77	73±3	69±.3	50±3	26±3
Bundle interval	Absolute, micra....	490	580±50	490±50	380±30	190±20
	Relative, per cent. } of cortex thick- ness	93	73	78	81	119
	Relative, per cent. } of stem diame- ter	—	6.0	6.2	6.8	10.6

It is clear from the last paragraph that there is a pronounced agreement between all the anatomical values and the water supply afforded to the plants, this relation being direct in all cases excepting that of bundle interval when this is expressed relatively to cortex thickness or stem diameter, in which cases the relation is inverse. The single submerged-leaf plant furnishes a general exception, which is not surprising, since all of its growth processes evidently had been greatly disturbed by complete submergence, as has been pointed out (see page 227).

## LEAVES

The leaf features studied comprised two petiolar characters and eight laminar characters. The petiolar features were the number of bundles and the width of the petiole; they pertain only to the root-leaves. The eight characters of the leaf-blade were studied for each of three different regions of the plant, or three leaf classes,—root-leaves, lower stem-leaves and upper stem-leaves. The root-leaves were of the basal rosette, the lower stem-leaves



FIG. 6. Typical representatives of four of the five groups of plants of *Ranunculus sceleratus*, first generation, as they appeared on September 15, 1915; C, amphibious; D, mesophyte; E, subxerophyte; F, xerophyte.

were from just above the rosette, and the upper stem-leaves were those attached just below the flower first to open. These are apparent in the photograph of figure 6. The eight laminar features were: the laminar area (one surface only), the mean frequency of stomatal distribution (on the upper and on the lower laminar surface), the number of cell layers of the chlorenchyma and of each of its two components, the spongy and palisade tissues, and the thickness of the chlorenchyma and of the external layer of palisade cells, this last being determined absolutely and relatively to the thickness of the chlorenchyma. The manner of obtaining the numerical data for

these various leaf features will first receive attention, then the data obtained will be presented, after which the generalizations derived from the data will be brought forward.

Only mature leaves were examined. Samples for sections of the petiole were secured midway between the junctions with the blade and with the stem. The petioles of the root-leaves were found to consist mostly of aerenchyma. The number of bundles and the width of the petiole were determined from cross sections.

From each blade a sample for sections was removed with a dissecting scalpel, being taken from the region between the main rib of a lateral lobe and the end of the adjacent deep sinus (see fig. 7). Such samples, when they were

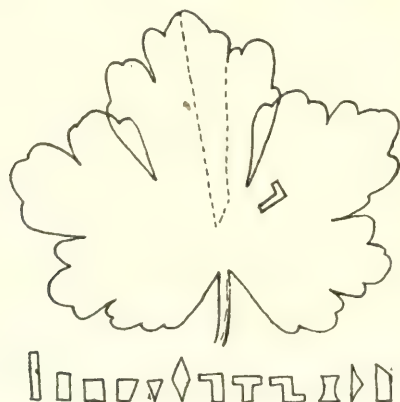


FIG. 7. Outline of a leaf of *Ranunculus sceleratus*, showing place from which the sample for stomatal counts (inside the dotted line) and that for subsequent paraffin sectioning (the L-shaped area) were taken. The latter sample was always obtained with a dissecting scalpel and the portions taken were of different shapes for different leaves whose samples were to be preserved in the same container, these shapes being shown here below the leaf outline.

to be preserved in the same container, were of different shapes for different leaves, these shapes being shown in figure 7 below the outline of the leaf blade. Thus a number of samples, or of sampled blades, could be kept together without confusion as to their identity or origin. The sampled blades were preserved in a 4-per cent. solution of formaldehyde for several weeks. Tracings were made of the preserved leaves and planimeter readings of the tracings were taken later. It should be remarked that these leaves were in the wilted condition when traced and that their area may be considerably less than in the case of the same leaves when turgid. But this point should not seriously interfere with such interpretations as are made from the areal values in the present study.

As soon as the outline of a leaf was traced, a strip was removed alongside, but not including, the central main rib from the tip of the central lobe to the junction of the three main veins (see fig. 7). This strip was examined for stomatal frequency without further preparation. The tissue had already been bleached by exposure to sunlight in the formaldehyde solution, so that direct stomatal counts were possible, by transmitted light. With an ocular magnifying 7.5 diameters and a 4-mm. objective, it was possible to secure a microscopic field having an area very close to 0.1 sq. mm. About ten different fields were examined, nearly equally distributed along the strip described above. The minimum, average, and maximum number of stomata per square millimeter were determined for each sample strip and for each of the two leaf surfaces, upper and lower. The stomatal counts were restricted to one part of the leaf as a general precaution, in spite of the fact that preliminary examination of different laminar regions did not disclose any variation corresponding consistently with location. Examination of leaves of various ages indicated that stomata appeared when the leaf area had reached about one tenth of the magnitude it would have when the leaf was mature, and that, after their appearance, their frequency of distribution decreased until leaf enlargement ceased.

The number of rows of palisade cells and of sponge cells appearing in each laminar cross section were counted, and the thickness of the external row of palisade cells, as well as that of the whole chlorenchyma zone (palisade and spongy tissues together) were determined. Often the row of cells appearing in the cross section as next interior to the external row of palisade cells proved to be partly palisade and partly sponge cells. Although the proportions of these two kinds of cells in this layer were of course not exactly equal, the number of palisade rows was taken as 1.5 in such cases. The thickness of the external row of palisade cells was expressed both absolutely (in micra) and as a percentage of the thickness of the entire chlorenchyma.

The various leaf data obtained are mostly presented by the diagrams of figures 8 to 12, in which the arrangement follows the scheme employed in figures 4 and 5. Each figure embodies the data for a single one of the five plant groups, excepting that the one plant available for the submerged-leaf group ultimately became amphibious, as has been described (see p. 221), so that the later observations on this plant are set down in figure 8 as submerged-leaf-amphibious (SA). In figure 13 are assembled the group means which are to be found in the lower parts of figures 8 to 12. Group letters and plant numbers are noted at the left of the diagrams, as well as the dates of observation. In the column at the right of the dates of observations there is indicated, by number or letter, just what leaves were examined. In this column a number or an interrogation point represents a certain root-leaf, while an average for a group of root-leaves is indicated by the letter "R." The sign "LS" denotes a lower stem-leaf or an average for a group of such leaves. The

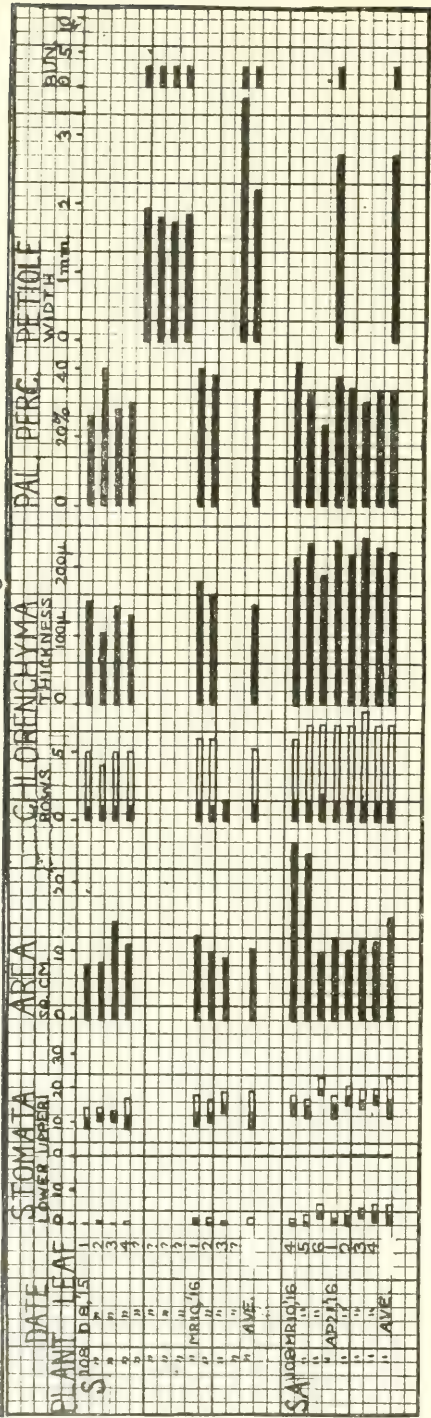
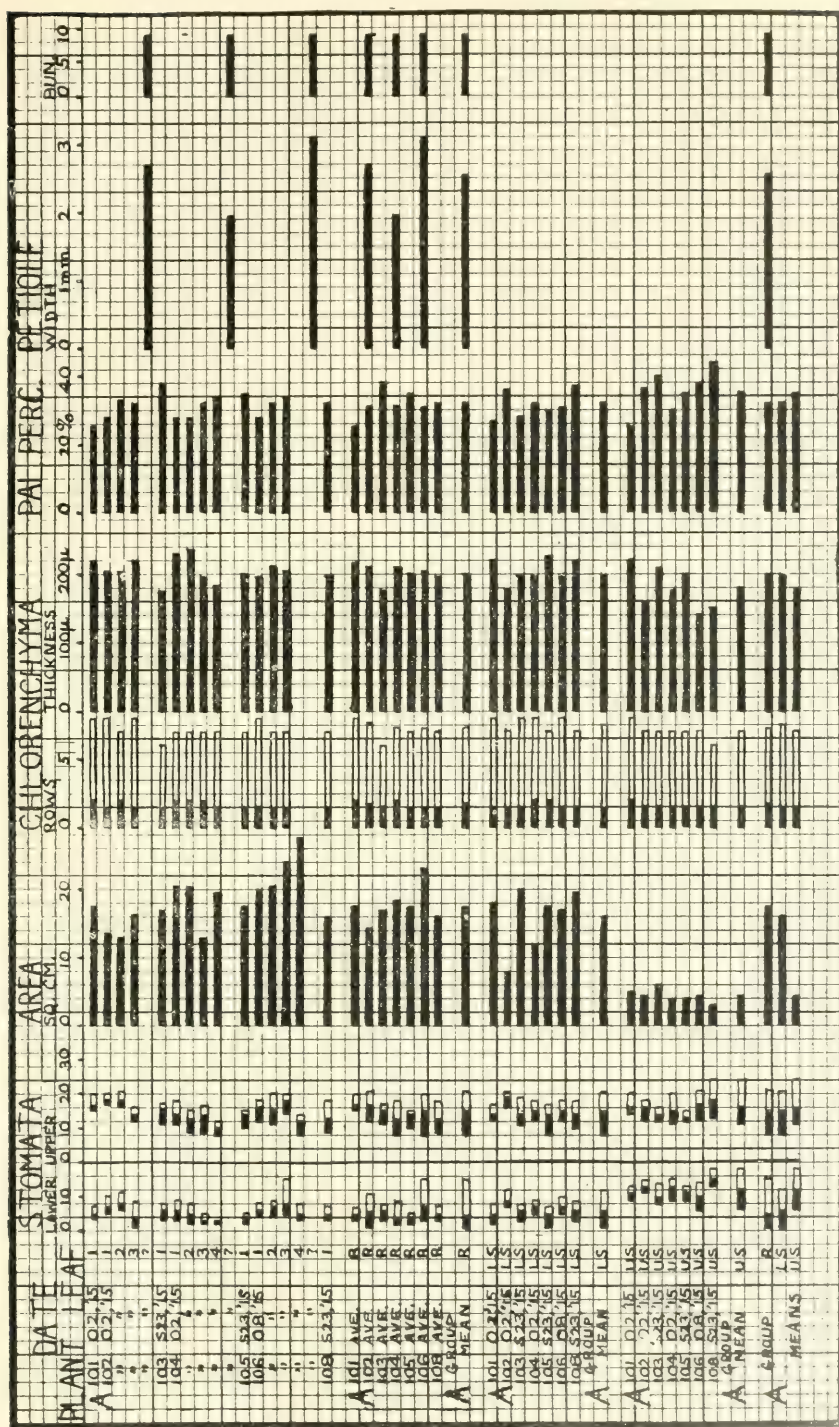


FIG. 8. Diagram showing numerical data for leaf features of the *submerged-leaf* culture of the first generation (1915) of plants of *Ranunculus sceleratus*. For description see text, p. 233. Compare with figures 9-13.



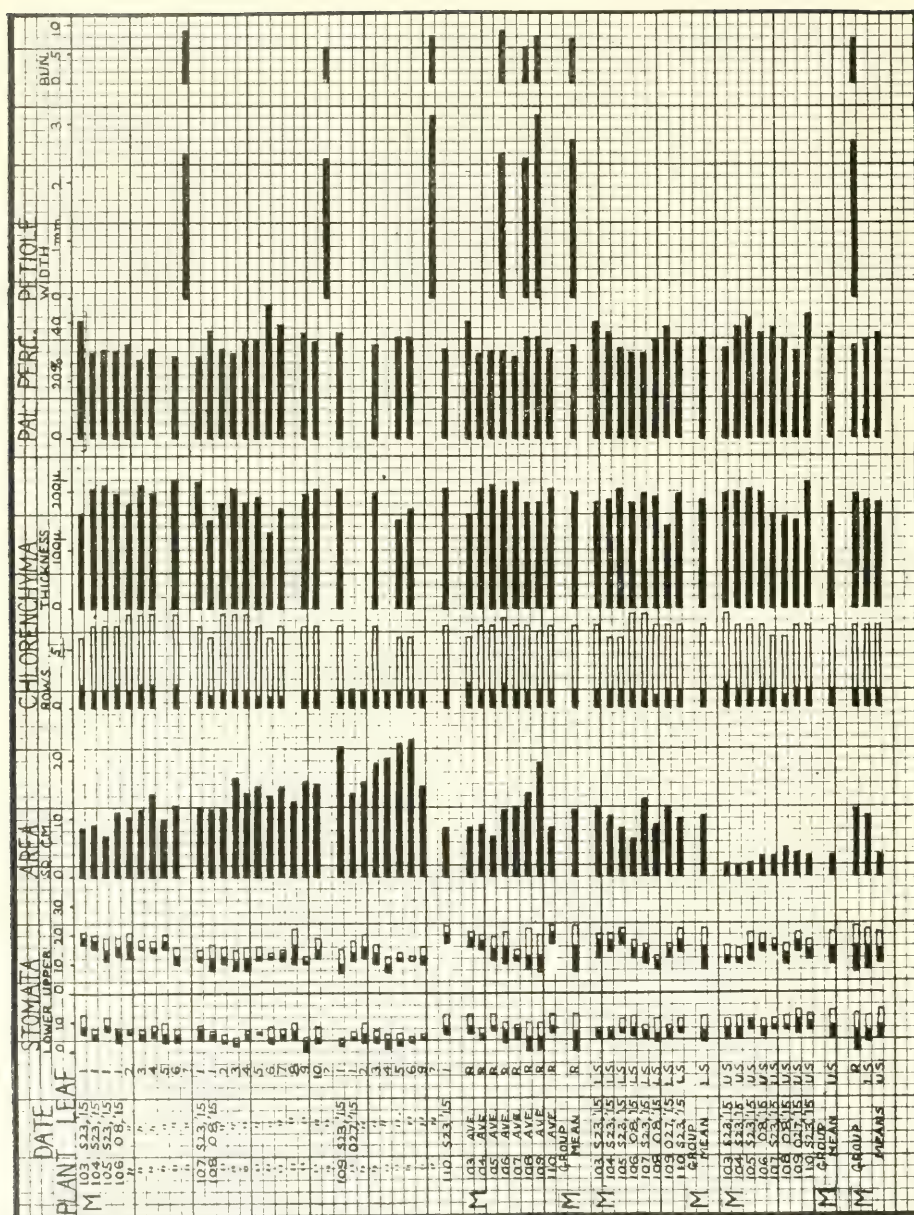


Fig. 10. Diagram showing numerical data for leaf features of the mesophyll group of cultures of the first generation (1915) of plants of *Ranunculus sceleratus*. For description see text, p. 233. Compare with figures 8, 9, 11, 12 and 13.

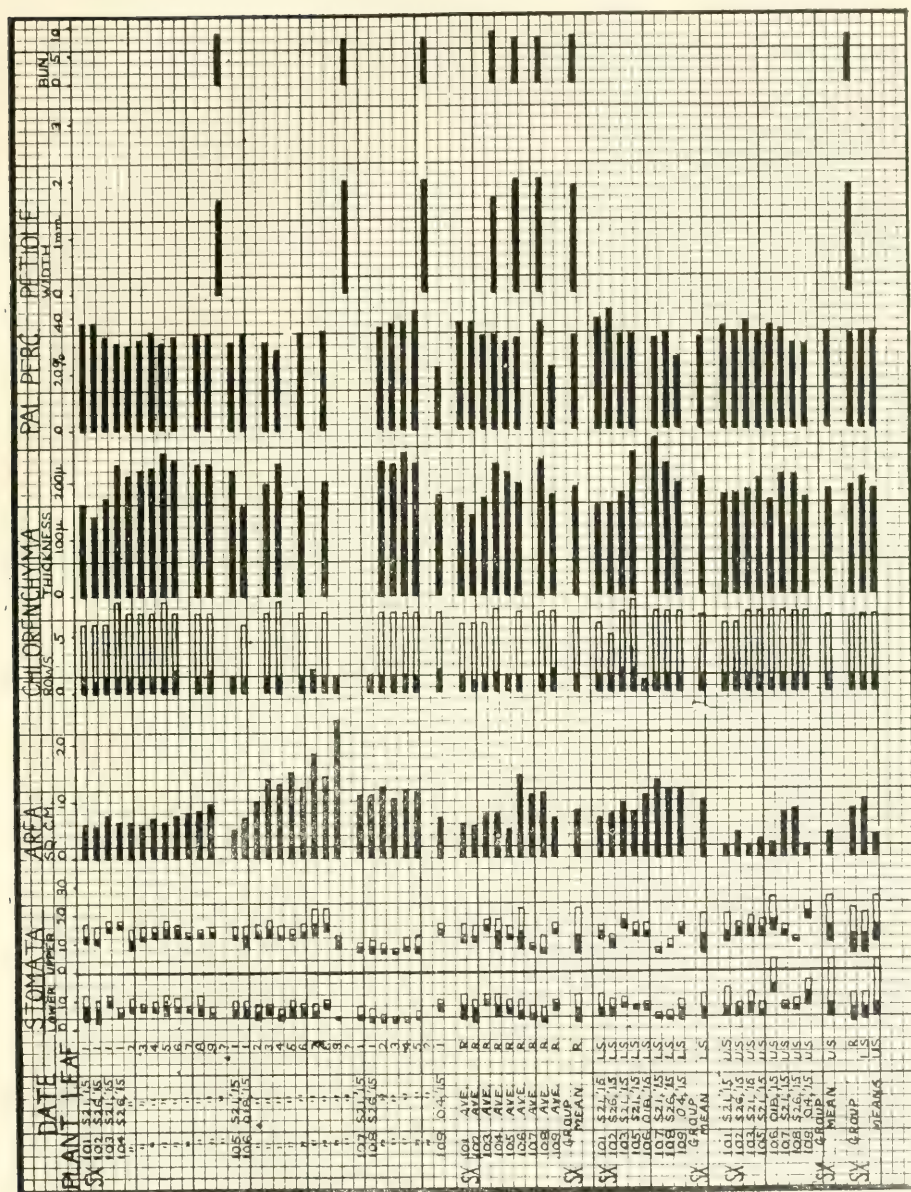


FIG. 11. Diagram showing numerical data for leaf features of the *subxerophyte* group of cultures of the first generation (1915) of plants of *Ranunculus sceleratus*. For description see text, p. 233. Compare with figures 8, 9, 10, 12 and 13.

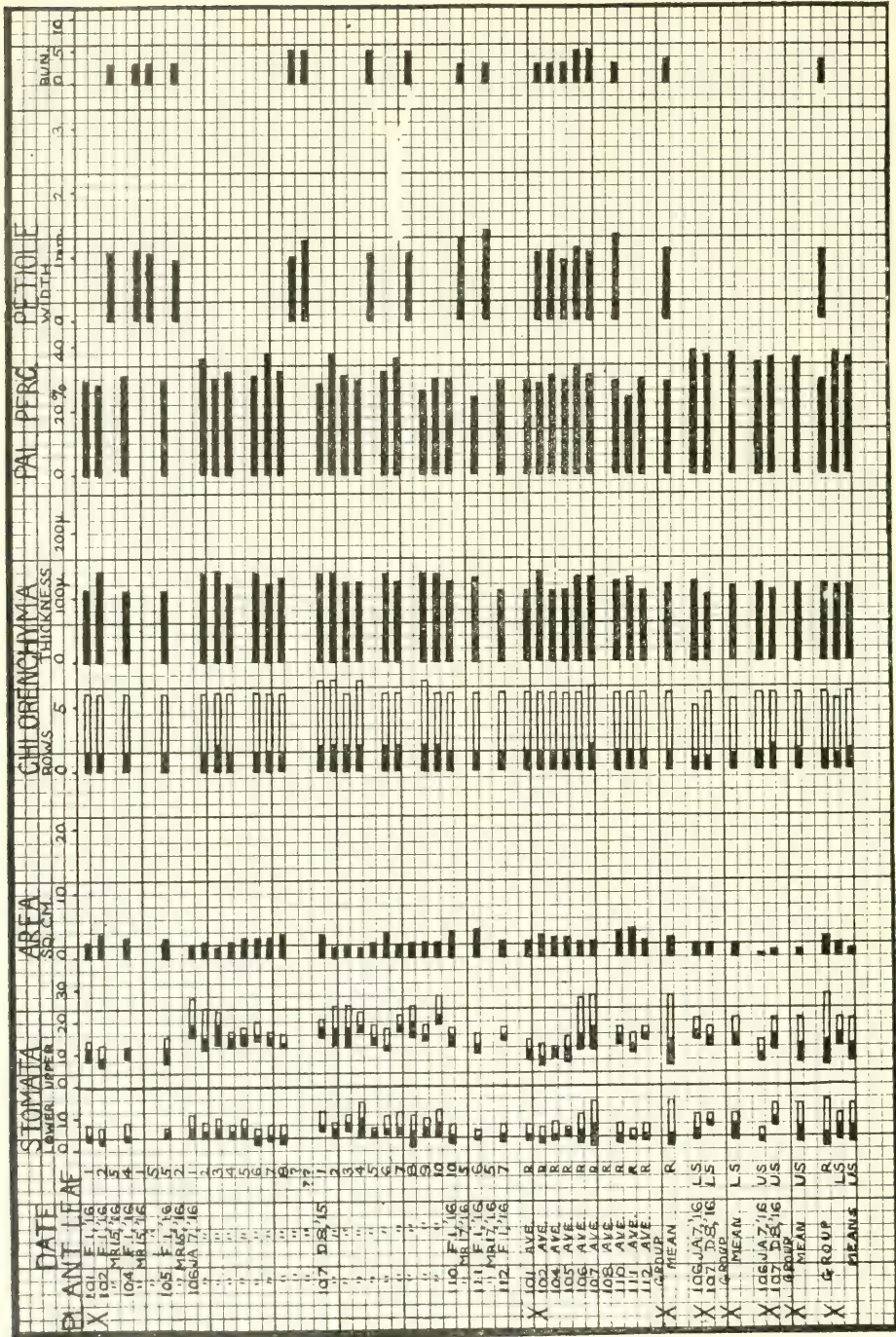


FIG. 12. Diagram showing numerical data for leaf features of the xerophyte group of cultures of the first generation (1915) of plants of *Ranunculus sceleratus*. For description see text, p. 233. Compare with figures 8, 9, 10, 11 and 13.

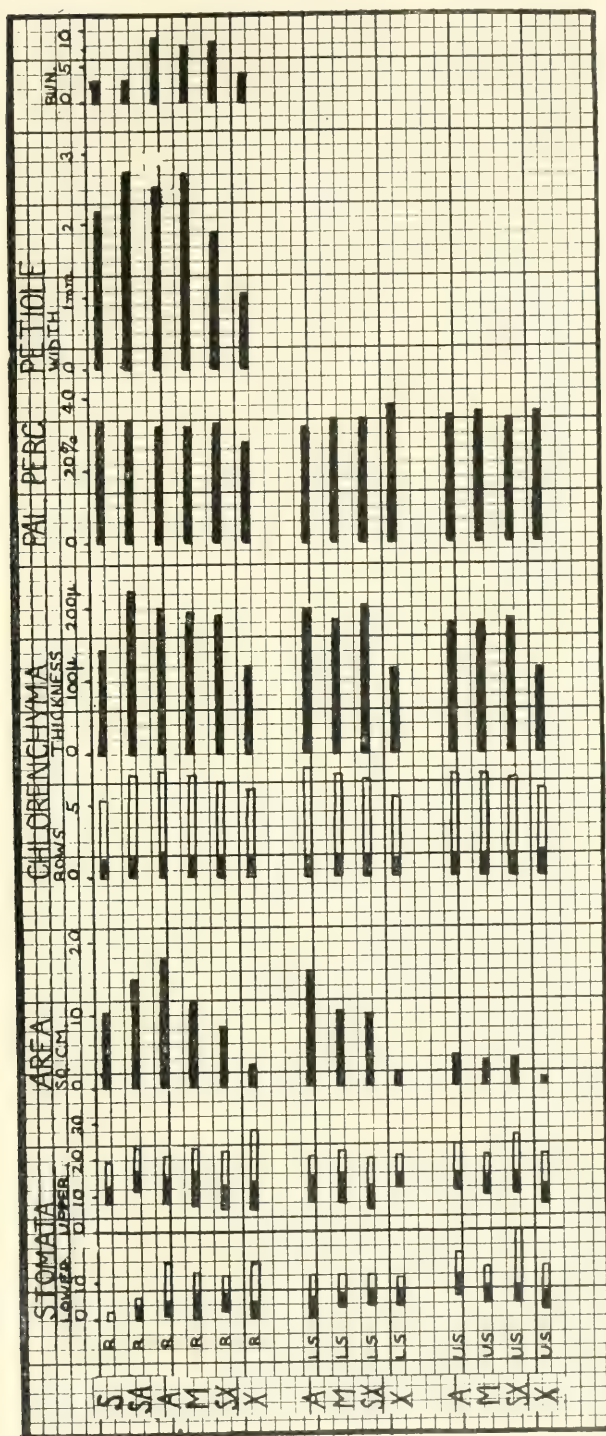


FIG. 13. Diagram showing group means of numerical data for leaf features of the first generation (1915) of plants of *Ranunculus sceleratus*, the plants grown with five different environmental complexes. For description see text, p. 233. Compare with figures 8-12, in which are shown the numerical data and means for the respective groups of cultures.

sign "US" represents, likewise, upper stem-leaves. When two or more root-leaves from one plant were examined, the average of their measurements is given below as a plant average (see "A101 Ave. R" in figure 9), and such plant means are averaged in their turn, to give the group mean. The plant features are designated above the diagrams, along with their respective scales of units. The word "Stomata" denotes average number of stomata per 0.1 sq. mm. The thickness of the external layer of palisade cells is denoted on these diagrams by the sign "Pal. Perc.," and the data given are expressed as percentage of the corresponding thickness of the whole chlorenchyma layer, in each case. The sign "Bun." indicates number of bundles in the petiole. As before, the lengths of the horizontal bands of these diagrams represent the magnitudes dealt with. In the case of the bands for frequency of stomatal distribution, the shaded portion represents the range between the minimum value obtained from the counts and the mean value, while the entire band represents the range between the minimum and the maximum values obtained from the counts. Of the bands for number of layers of chlorenchyma cells, the shaded portion of each band represents the number of rows of palisade, and the entire band stands for the whole chlorenchyma, the length of the unshaded portion corresponding to the number of rows of sponge cells as seen in the cross section of the leaf.

The group averages from the data given in the diagrams of figures 8 to 13 are shown in table IV, where the arrangement corresponds with that followed in tables II and III. Maxima are again in full-face type, minima in Italics. Each of these group averages (except those for the submerged-leaf plant) was secured from data obtained from two or more different plants, as is shown by the diagrams of figures 8 to 13.

As is shown in the diagrams of figures 8 to 12, the leaves used for measurements were secured in September and October of 1915, for the amphibious, mesophyte, and subxerophyte groups, but not until from December, 1915, to March, 1916, for the submerged-leaf and xerophyte groups. This suggests that difference in season (with the concomitant differences in the climatic complex) rather than difference in water supply may have caused certain differences between the submerged-leaf and xerophyte groups, on the one hand, and the remaining ones, on the other. This idea is supported by measurements made of an automatically watered plant during February and March, 1916.

The soil-water content of this culture varied from 20.3 to 23.2 per cent. and therefore was midway between that of the mesophyte and that of the subxerophyte group (see p. 224). The leaf features of this automatically irrigated culture may be compared with those of the mesophyte and subxerophyte groups in the diagram of figure 14. The arrangement, signs, etc., in this figure are like those in figures 8 to 12. The measurements of the root-leaves, given serially in the order of the appearance of the latter on the stem,

TABLE IV

*Average numerical data for leaves of Ranunculus sceleratus of the first generation (1915), the plants grown with five different environmental complexes*

ANATOMICAL FEATURE		SUB-MERGED GROUP	AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXERO-PHYTE GROUP	XEROPHYTE GROUP		
Root-leaf petioles	Number of bundles..	3.0	9.0±0	7.7±.7	8.3±.3	3.7±.3		
	Width, mm.....	2.2	2.6±.2	2.7±.2	1.9±.1	1.1±.04		
Laminar area, sq. cm	Root-leaves.....	10.5	17.7±.6	11.3±1.0	8.4±.8	3.1±.2		
	Lowest stem-leaves..	—	16.0±.1	10.3±.6	9.9±.5	2.0±0		
	Upper stem-leaves...	—	4.4±.2	3.4±.2	4.0±.7	0.8±.2		
Stomatal frequency per 0.05 sq. mm.	Root-leaves, lower surface.....	0.4	2.6±.2	3.6±.2	3.6±.2	2.7±.1		
	Root-leaves, upper surface.....	6.3	7.4±.2	8.0±.4	6.3±.3	6.8±.4		
	Lowest stem-leaves, lower surface.....	—	3.2±.2	3.6±.1	3.4±.2	3.8±.6		
	Lowest stem-leaves, upper surface.....	—	7.5±.2	7.9±.3	6.4±.5	8.0±.4		
	Upper stem-leaves, lower surface.....	—	5.9±.3	4.6±.2	4.5±.4	3.5±1.2		
	Upper stem-leaves, upper surface.....	—	7.8±.2	7.6±.3	7.8±.3	6.5±1.3		
Chlorenchyma, root-leaves	Entire	No. of layers.....	5.2	7.2±.2	6.9±.1	6.6±.2	6.6±.04	
		Thickness, micra....	145	201±3	194±5	190±9	121±3	
	Palisade por-tion	No. of layers.....	1.1	1.7±.1	1.6±.1	1.5±.1	1.6	
		Thickness <sup>a</sup>	Absolute, micra..	48	64±1	63±1	63±4	35±1
			Relative, per cent. of entire chlor-enchyma.....	34	32±1	32±1	33±1	29±1
Chlorenchyma, lowest stem-leaves	Entire	No. of layers.....	—	7.6±.2	7.0±.2	6.7±.2	5.5±.4	
		Thickness, micra....	—	203±5	184±4	206±13	115±9	
	Palisade por-tion	No. of layers.....	—	1.6±.1	1.4±.04	1.6±.1	1.3±.2	
		Thickness <sup>a</sup>	Absolute, micra..	—	64±2	63±2	70±4	44±5
			Relative, per cent. of entire chlor-enchyma.....	—	32±1	34±1	34±1	38±1

<sup>a</sup>Thickness here concerns only the external layer of palisade; see p. 231.

TABLE IV—*Continued*

ANATOMICAL FEATURE			SUB-MERGED GROUP	AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXEROPHYTE GROUP	XEROPHYTE GROUP
Chlorenchyma, upper stem-leaves	Entire	No. of layers.....	—	7.0±.1	6.9±.1	6.8±.1	6.0±0
		Thickness, <i>micra</i> ....	—	182±9	181±6	187±5	115±4
	Palisade portion	No. of layers.....	—	1.6±.1	1.5±.04	1.4±.04	1.8±.2
		Thickness <sup>a</sup>	—	63±4	65±4	63±2	41±2
		Relative, <i>per cent.</i> of entire chlorenchyma.....	—	35±2	36±1	34±1	36±1

<sup>a</sup>Thickness here concerns only the external layer of palisade; see p. 231.

are shown in the upper part of the figure, while below these is given the average for all the leaves of the culture, followed by the averages for the mesophyte group ("M"), for this culture ("Au3"), and for the subxerophyte group ("SX").

A comparison of these three averages will show that, for the automatically watered culture, the values for stomatal frequency on either surface, thickness of chlorenchyma, and number of petiolar bundles are markedly lower, in each case, than are the corresponding values for the mesophyte and subxerophyte groups. This makes it quite probable that similarly lower values shown in regard to these features by the xerophyte and submerged-leaf groups (see figure 13, table IV, and the following discussion) were lowered by difference in season rather than by difference in water supply. The water supply may have been only indirectly responsible because of its lengthening the growth period so as to make it extend through different seasons.

Examination of table IV and of the group means in figure 13 brings out the following generalizations regarding the various anatomical features of the leaves. The *number of bundles* in the *root-leaf petiole* was largest in the amphibious group and nearly as large in the subxerophyte and mesophyte groups, while it was relatively very small in the submerged-leaf and xerophyte groups. The *width of the root-leaf petiole* was greatest for the amphibious and mesophyte groups and very small for the submerged-leaf and xerophyte groups, being smallest for the latter. (It may be noted here that the petiolar width for the emerged leaves of the single submerged-leaf plant is the greatest value being over 2.7 mm.)

The *area of the leaf blade* for *root-leaves* was greatest for the amphibious group and progressively smaller with progressive decrease in the water supply, and was also smaller with submergence. This area for *lowest stem-leaves* was also greatest for the amphibious group and progressively smaller with increasingly lower water supply. The *laminar area* for *upper stem-leaves* was

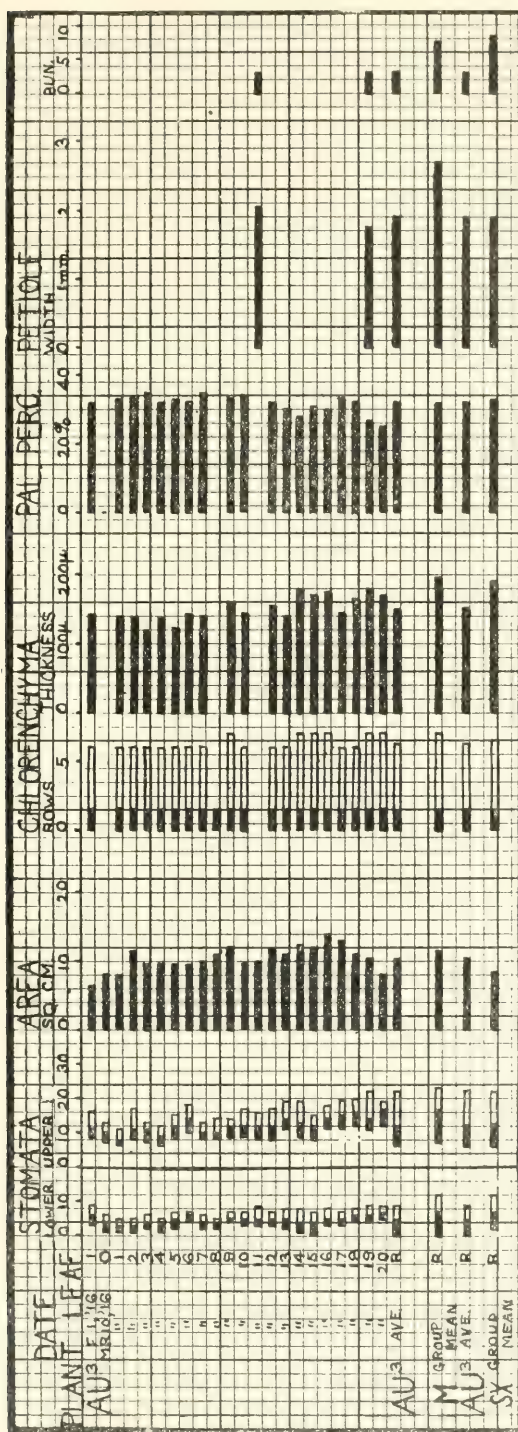


FIG. 14. Diagram showing numerical data for leaf features of the automatically irrigated culture of the first generation (1915) of plants of *Ranunculus sceleratus*. For description see text, p. 240. Compare with figures 8-12.

smallest for the xerophyte group and relatively very large for the other three groups for which data are available, being greatest for the amphibious and subxerophyte groups.

*Stomatal frequency* for the *lower surface* of *root-leaves* was greatest for the mesophyte and subxerophyte groups, of medium magnitude for the amphibious and xerophyte groups, and smallest for the submerged group. It was, for the *upper surface* of these same leaves, greatest for the mesophyte and amphibious groups, and smaller (but about alike) for the other three groups. Stomatal frequency for the *lower surface* of *lowest stem-leaves* was about the same for all four groups considered. For the *upper surface* of these leaves, it was smallest for the subxerophyte group and not far from alike for the other three groups. Stomatal frequency for the *lower surface* of the *upper stem-leaves* was largest for the amphibious group and smaller (and similar) for other water supplies. For the *upper surface* of these leaves, it was somewhat smaller for the xerophyte group than for the other three groups considered, for which it was about alike.

The *number of layers of chlorenchyma cells* in *root-leaves* was greatest for the amphibious and mesophyte groups and somewhat smaller with submerging and with lower water supply. The *number of layers of palisade cells* in these leaves was lowest with submerging, but about alike for the four non-submerged groups. The *thickness of the chlorenchyma* in root-leaves was about the same for the amphibious, mesophyte, and subxerophyte groups and smaller for the other two, being least for the xerophyte group. The same generalization holds for the *absolute thickness* of root-leaf *palisade*; *relatively* to that of the entire chlorenchyma this thickness was smallest for the xerophyte group, and somewhat greater for the other four.

The *number of layers of chlorenchyma cells* in the *lowest stem-leaves* was greatest for the amphibious group and progressively smaller with progressively lower water supply. The *number of palisade layers* in these leaves was greatest (and about alike) for the amphibious and subxerophyte groups, and somewhat smaller for the other two. The *thickness of chlorenchyma* in the lowest stem-leaves was greatest for the subxerophyte and amphibious groups, somewhat smaller for the mesophyte and smallest for the xerophyte group. The *absolute palisade thickness* in these leaves was smallest for the xerophyte group and about alike for the other three groups for which data are available; *relatively* to the entire chlorenchyma thickness, this value was greatest for the xerophyte group and about alike for the other three.

The *number of chlorenchyma layers* in the *upper stem-leaves* was of about the same magnitude for the amphibious, mesophyte and subxerophyte groups and considerably smaller for the xerophyte group. The *number of palisade layers* in these leaves was largest for the xerophyte group and somewhat smaller for the other three. The *chlorenchyma thickness* in the upper stem-leaves was least for the xerophyte group and about the same for the other

three. The same generalization is true for the *absolute thickness of the palisade* in these leaves; *relatively* to that of the entire chlorenchyma this thickness was about the same for all four groups considered.

From the generalizations just given, and from the data from which they were derived (figures 8-13 and table IV), it is clear that *the three classes of leaves* here dealt with (root-, lowest stem- and upper stem-leaves) all agreed in their relations to the environmental conditions of the various groups only in showing the lowest value with the xerophyte group in regard to each of three features; viz., number of layers of chlorenchyma present, thickness of chlorenchyma, and absolute thickness of the external layer of palisade. As has been pointed out, this difference between the xerophyte group and the others may not be due directly to difference in water supply. The *root-leaves* and *lower stem-leaves* agreed in their relations to external conditions only in regard to *laminar area*, this being uniformly greatest for the amphibious plants and progressively smaller with lower water supply. No other agreement between the classes of leaves was discovered, besides those just set forth. (It should be noted that only one class, root-leaves, was examined for the submerged-leaf condition.)

The *amphibious* group was foremost, or among the foremost groups, in regard to all features except two. These two exceptions were the frequency of stomatal distribution on the lower surface of the root-leaves (for which the value was highest with the mesophyte and subxerophyte groups) and the relative thickness of palisade of the lowest stem-leaves (for which the value was highest with the xerophyte group). It was foremost, with the values for the other groups decreasing to correspond with the decrease in water supply, in regard to the *laminar area of root-leaves* and of *lowest stem-leaves*, and the *number of layers of chlorenchyma cells of the lowest stem-leaves*. Submerging, in comparison with the amphibious condition, was accompanied by a lowering of the values in regard to all the features for which data are available, with the exception of the thickness of the external palisade row relative to the thickness of the entire chlorenchyma. But, as has been pointed out, some of these differences may have been directly due to seasonal differences.

#### FLOWERS AND SEEDS

During the earlier part of the period of flowering, the flowers were pollinated by hand as they opened from time to time, care being taken that the pollen used was always derived from the same culture group as the pistils to which it was applied. When the achenes were mature (as judged by the ease with which a light pressure removed them) they were removed and dried in the air for a few days. Then they were kept in glass vials, these being inclosed in a can, and were treated occasionally with vapor of carbon bisulphide. A preliminary examination of preparations of the floral structures

disclosed no histological differences between those of different culture groups. The youngest stages that were examined showed the appearance of the primordia of the petals and stamens, but not yet those of the pistils. In the oldest stages the achenes were falling, and the embryos were small, with only the adjacent part of the endosperm digested.

The features studied more carefully in regard to reproduction were: number of pistils in the first and in the tenth flower to open, upon each plant, and viability of the seeds produced. The number of pistils per flower was obtained by the counting of the fruits produced, both normal and aborted. A hundred achenes, chosen at random from the vial for each culture, were

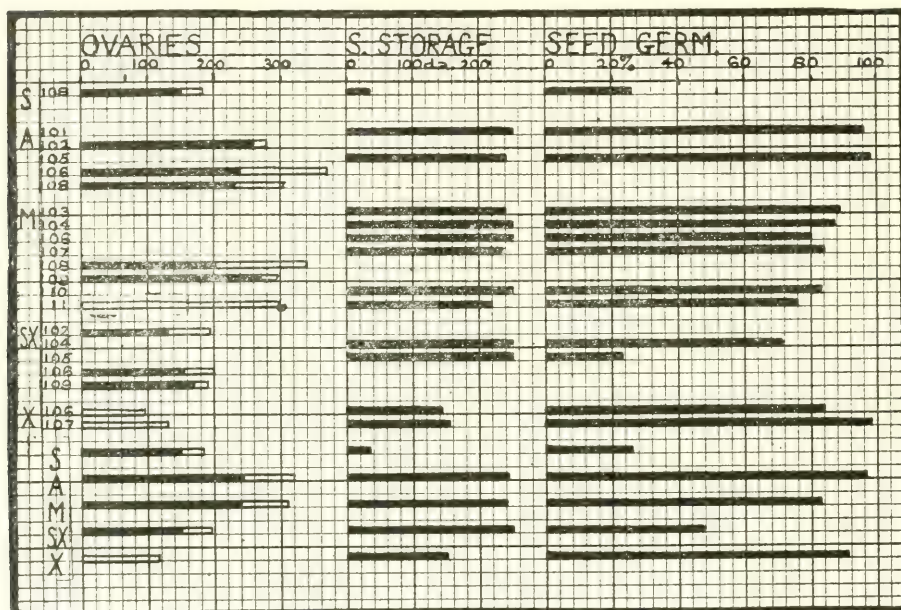


FIG. 15. Diagram showing numerical data for reproductive structures of the first generation (1915) of plants of *Ranunculus sceleratus*, the plants grown with five different environmental complexes. For description see text, p. 246.

germinated in the viability tests. These were sown in sand kept with a uniform moisture content, about the same as for the soil of the mesophyte group. The records show the number that germinated in each test.

The data obtained by these determinations are diagrammatically set forth in figure 15, which also shows the number of days that elapsed between the first anthesis and the germination test, in each case ("S. Storage"). On this diagram the group letters and plant numbers occur at the left, as in earlier diagrams, and the lower part of the diagram gives the group means, with

their letters. The plant features dealt with are indicated above, each with its own scale of units. The column of bands headed "Ovaries" shows the number of achenes obtained from the first flower to open (total length of the band) and the number obtained from the tenth flower to open (shaded portion).

The group means are tabulated in table V, which is self-explanatory, maxima and minima being again shown by full-face type and by italics, respectively.

TABLE V

*Average numerical data for reproduction in plants of Ranunculus sceleratus of the first generation (1915), the plants grown with five different environmental complexes*

PLANT FEATURE		SUB-MERGED-LEAF GROUP	AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXEROPHYTE GROUP	XEROPHYTE GROUP
Number of achenes per flower	First flower to open	185	<b>318</b> ±21	310±12	195±2	<i>115</i> ±15
	Tenth flower to open	<i>150</i>	<b>243</b> ±7	238±13	152±9	—
Viability of achenes ( <i>per cent.</i> germinating).....		26	<b>97</b> ±1	83±1	47±20	<i>91</i> ±6

The number of achenes per flower was largest for the amphibious and mesophyte groups, lower with submerging of the leaves, and progressively lower for the subxerophyte and xerophyte groups. Seed viability was also greatest for the amphibious group, but nearly as great for the xerophyte group. It was very low for the submerged-leaf group and was progressively lower in the series from amphibious to subxerophyte. There was apparently no relation between seed viability and the length of time during which the seeds had been stored (see fig. 15).

## EXPERIMENTS AND RESULTS WITH RANUNCULUS SCLE- ERATUS, SECOND GENERATION (1916)

### GENERAL CONSIDERATIONS

The plants used for these experiments were from seedlings obtained in the viability tests of the seed from the first generation. As has been stated, the flowers producing these seeds had been pollinated, in each case, with pollen from plants in the same culture group. When a few days old, these seedlings were transplanted from the germination tests, each to its culture pot of the second series. The soil used in these pots was the mixture of garden soil and white sand already described (see p. 216), and the moisture conditions for each seedling were of the same group as had been those for its parents. The seedlings were transplanted to their culture pots on May 16, and a few that died shortly after were replaced by others from the germination tests. The single submerged-leaf plant of the first generation had not

flowered until May 5, 1916, as has been mentioned, so that its achenes were sown much later, and six seedlings were transplanted and submerged on August 1.

For the amphibious, mesophyte and subxerophyte groups, the purely vegetative period ended with the production of the first flower, at dates between July 22 and August 7. Most of the plants of the xerophyte group succumbed before August 17, and the survivors had not yet flowered by April 27, 1917, when the experimentation was discontinued. Three of the submerged-leaf group died during the fall and winter, and the other three had produced only rosettes of small leaves by April 27, 1917. Consequently only the amphibious, mesophyte and subxerophyte groups of plants could be completely compared in this series. The soil-moisture content was, on the average, 47.6 per cent. for the amphibious group, 32.6 per cent. for the mesophyte group, 13.5 per cent. for the subxerophyte group, and 6.3 per cent. for the xerophyte group, this value always calculated on the basis of the *dry weight* of the soil. When these means are compared with the first, that of flooded soil, taken as a standard they are found to have the relations, 100:68:28:13.

As has been mentioned in the introduction of the present paper, F. E. Clements [1905] distinguished nine distinct forms of *Ranunculus sceleratus*, differing in many ways, when the plants were grown under various conditions at Lincoln, Nebraska. The present studies failed to disclose nearly so many forms, and the differences here described relate to comparatively few characteristics. There are considerable differences between the two climates (of Lincoln and Minneapolis), in regard to light at least, the sun's altitude being greater at Lincoln; and the greenhouse roof at that station was much less subject to soiling by soot from passing locomotives, which was rather pronounced at Minneapolis. It thus appears possible that the reduction in the number of forms and in the differences between them may have been related, in part, to the lower solar altitude and to the greater opaqueness of the greenhouse roof at Minneapolis. With this idea in mind, the second generation of plants in these studies were still further shaded. While the dirty glass reduced the actinometric index (with photographic paper) at midday with clear sky, from 1.00 to 0.23, the paint on the roof reduced it to 0.035 for the second generation of plants.

## RESULTS

Not all the anatomical and histological features measured and studied for the first generation were dealt with in the second series; selection was made of those features that seemed most likely to throw light on the question above suggested, as to whether increased shading in the greenhouse might decrease the differences observed between the different groups of plants. Root features were not considered at all. Stem measurements were made, as for the former

series, of height (total and to the first flower), basal diameter, thickness of cortex near the base, and thickness of aerenchyma (absolute and as percentage of the whole thickness of the cortex). For the leaves, only the areas (one side) were determined, and the measurements were obtained in a somewhat different manner from that followed for the first generation. Four representative mature leaves, of average size by visual estimate, were removed from each plant, one on each of the dates July 8, 15, 25 and 31, and the areas of these were afterwards determined. Thus four areal measurements are at hand for each plant, which were averaged to give the plant mean in each case. These plant means were then averaged for all the plants in the given group, to give the respective group means. Each plant was artificially pollinated with its own pollen and the achenes were counted (for the first and tenth flowers to open) and all were collected and tested for viability, as in the earlier series.

The data obtained are set forth in table VI, which also gives the source of the seed (seed-producing parent), date of transplanting and of first flowering, and the soil-moisture values, in each case.

An examination of the individual measurements given in table VI reveals no correlation within the several groups, except that it appears that the smaller the leaf area was, the later was the occurrence of first flowering, and the smaller was the basal diameter of the stem. Neither the parentage of the plants nor their positions in the greenhouse seems to have influenced the individual variations from the means of the respective groups.

The group averages and their probable errors, such as have been employed in the discussion of the results of the earlier series of experiments, are given in table VII, these group means being derived from the detailed data of table VI. Also, to make later comparison easier, the corresponding average values for the first generation are repeated in table VII. Maxima and minima are indicated as heretofore. The leaf-area averages for the second generation each represent about 40 leaves.

From the data for the second generation (table VII) the following generalizations may be pointed out. It is clear that marked differences between the amphibious, mesophyte and subxerophyte groups are not exhibited for either of the *height* values nor for the *basal diameter of the stem*, but that the other features studied all show more or less pronounced differences. In the case of *thickness of stem cortex* the data for these three culture groups show the highest value for the amphibious group and markedly lower ones for the other two, which are about the same. For *thickness of stem aerenchyma*, both *absolute* and *relative to that of the entire cortex*, the maximum occurs for the amphibious group, with progressively lower indices corresponding to progressively lower supply of moisture to the plants; the minimum occurs for the subxerophyte group. As to *leaf area*, it appears that the mesophyte and subxerophyte groups were sensibly alike in this respect, but that the areal

TABLE VI  
Data concerning the second generation of *Ranunculus sceleratus*, the plants grown with four different environmental complexes

ENVIRONMENTAL COMPLEX	PLANT NUMBER	SEED-PRODUCING PARENT	DATE OF TRANSELANATING	DATE OF FIRST FLOWERING	AVERAGE SOIL-MOISTURE CON- TENT (PER CENT. OF DRY WEIGHT)	STEM			THICKNESS OF AEREN- CHYMA		LEAF AREA					NUMBER OF PISTILS		SEED VIABILITY (PER CENT.)	
						Height	To first flower	Basal diameter	Thickness of cortex	Absolute	Relative, per cent. of cor- tex thickness	July 8	July 15	July 25	July 31	Average	In first flower		In tenth flower
cm.	cm.	mm.	mm.	mm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.	% cm.			

Amphibious group...	201	101	May 16	July 24	45.0	35	31	0.480	560.46	82	18.8	19.7	16.4	16.0	17.7	175	144	27
	202	101	May 16	July 23	45.6	37	31	0.510	560.47	84	17.7	17.9	19.16	8.18	1.1	177	122	
	203	103	May 16	July 22	48.5	40	33	0.600	580.51	88	18.7	22.6	24.5	21.1	21.7	213	152	
	204	104	June 5	August 6	52.0	40	33	0.540	610.49	80	12.9	22.8	23.1	19.2	215	149		
	205	105	May 16	July 23	46.6	37	31	0.580	720.68	94	19.2	20.6	20.7	17.5	19.5	181	137	76
	206	106	May 16	July 24	49.1	38	32	0.530	560.51	91	15.4	20.1	19.2	18.1	18.2	188	153	75
	207	105	May 16	July 25	53.5	39	33	0.520	580.47	81	18.6	18.4	14.7	18.7	17.6	182	136	
	208	108	May 16	July 25	43.6	38	33	0.540	680.54	79	15.8	21.1	20.9	19.8	19.4	181	152	42
	209	105	May 16	July 27	51.3	35	30	0.500	610.51	84	14.5	17.4	14.4	16.2	15.6	185	132	
	210	105	May 16	August 1	41.0	28	23	0.410	470.33	70	8.7	9.4	13.4	10.5	250	169		
Mesophyte group....	203	103	May 16	July 25	32.6	36	31	0.490	470.35	74	13.0	15.7	15.2	13.9	14.5	202	146	10
	204	104	May 16	July 23	31.5	34	28	0.630	490.35	71	14.8	17.1	16.6	19.5	17.0	197	170	
	205	105	May 16	July 29	31.1	33	28	0.450	470.40	85	11.5	14.5	16.7	12.8	13.9	180	163	42
	206	106	May 16	July 25	33.5	36	32	0.600	540.46	85	14.6	16.7	16.1	15.0	15.6	196	167	5
	207	107	May 16	July 28	30.7	32	26	0.470	440.33	75	11.1	16.2	17.3	17.1	15.4	197	181	
	208	108	May 16	July 26	32.9	34	28	0.480	470.37	79	11.7	14.8	16.2	13.0	13.9	208	181	
	209	109	June 5	August 7	34.0	33	29	0.380	440.32	73	9.1	10.6	11.6	12.3	10.9	213	149	
	210	110	May 16	July 27	32.2	29	24	0.460	510.39	76	11.1	13.7	14.4	13.1	13.1	176	148	26
	211	111	May 29	August 4	34.7	33	27	0.440	420.33	79	7.7	10.7	13.0	13.1	11.1	179	147	

Subxerophyte group	201	101	May 16	July 30	15.8	33	23	0.49	0.47	0.32	68	10.2	11.6	15.8	14.3	13.0	185	149	64
	202	102	May 16	August 3	16.7	31	23	0.45	0.46	0.33	72		7.8	11.3	14.9	11.3	206	154	
	203	103	May 16	July 22	12.6	37	30	0.61	0.40	0.28	70	14.6	15.6	18.8	15.8	16.2	247	198	
	204	104	May 16	July 25	18.0	38	31	0.58	0.47	0.35	75	10.6	15.4	22.3	20.5	17.2	241	217	
	205	104	May 16	July 23	15.3	37	30	0.57	0.42	0.30	71	12.4	18.2	18.4	15.4	16.1	232	174	85
	206	106	May 16	July 24	10.4	36	30	0.59	0.54	0.40	74	11.5	13.5	16.9	18.6	15.1	225	184	40
	207	107	May 16	July 24	9.0	36	28	0.59	0.46	0.33	72	12.0	17.3	17.8	20.0	16.8	214	188	
	209	109	May 16	July 24	13.0	35	27	0.53	0.42	0.33	79	7.7	13.2	15.8	15.9	13.2	193	165	89
	210	104	May 16	July 24	10.9	37	31	0.58	0.40	0.28	70	11.8	15.4	16.6	18.4	15.6	194	177	
	202	107	May 29		7.5							2.2	4.0			3.1			
Xerophyte group...	203	107	May 29		5.4								1.2	1.5		1.4			
	207	106	June 5		5.9							3.1	3.8	4.8		4.3			
	208	107	May 16											2.0		2.0			

TABLE VII

Average numerical data for stem, leaf, and reproduction features of plants of *Ranunculus seleratus* of the second generation (1946) and for corresponding features of the first generation (1945) of the same species

ANATOMICAL FEATURE		CULTURE SERIES	AMPHIBIOUS GROUP	MESOPHYTE GROUP	SCUMEROPHYTE GROUP
Height	Total, cm.	First generation	45 ± 1	39 ± 1	29 ± 1
		Second generation	37 ± 7 (-18) <sup>a</sup>	33 ± .4 (-15) <sup>a</sup>	36 ± .5 (+24) <sup>a</sup>
	To first flower, cm.	First generation	33 ± 2	28 ± 1	21 ± 1
		Second generation	31 ± .5 (-6)	28 ± .5 (0)	28 ± .8 (+33)
Basal diameter, mm.		First generation	9.7 ± .2	7.9 ± .2	5.6 ± .3
		Second generation	5.2 ± .1 (-47)	4.9 ± .2 (-38)	5.5 ± .1 (-2)
Thickness of cortex, micra		First generation	790 ± 50	630 ± 10	470 ± 10
		Second generation	590 ± 10 (-25)	470 ± 10 (-25)	450 ± 10 (-4)
Thickness of aerenchyma		First generation	580 ± 50	440 ± 0	240 ± 10
		Second generation	500 ± 10 (-14)	370 ± 10 (-16)	320 ± 10 (+33)
	Absolute, micra	First generation	73 ± 3	69 ± .3	50 ± 3
	Relative, per cent. of thickness of cortex	Second generation	83 ± 1 (+14)	77 ± 1 (+12)	72 ± 1 (+44)
Laminar area, root-leaves (sq. cm.)		First generation	17.7 ± .6	11.3 ± 1.0	8.4 ± .8
		Second generation	17.8 ± .5 (+1)	13.9 ± .4(+23)	14.9 ± .5 (+77)
Number of achenes per flower	First flower to open	First generation	318 ± 21	310 ± 12	195 ± 2
		Second generation	195 ± 5 (-39)	194 ± 3 (-37)	215 ± 6 (+10)
	Tenth flower to open	First generation	243 ± 7	238 ± 13	152 ± 9
		Second generation	145 ± 3 (-40)	161 ± 4 (-32)	178 ± 5 (+17)
Seed viability (per cent.)		First generation	97 ± 1	83 ± 1	47 ± 20
		Second generation	55 ± 10 (-43)	21 ± 6 (-75)	70 ± 9 (+49)

<sup>a</sup> The number in parentheses is the percentage of the first generation value by which that value is increased (+) or decreased (-) to give the second generation value.

value is significantly larger for the amphibious group. It was also markedly smaller for the xerophyte group, being  $2.7 \pm .5$ . The *number of achenes per flower, for the first flower to open*, appears to be practically the same for the amphibious and the mesophyte groups and somewhat greater for the subxerophyte group. But this generalization does not hold *for the tenth flower to open*, in which case there appears to be a marked inverse correlation between water supply and this anatomical value; the maximum occurs for the subxerophyte and the minimum for the amphibious group. Both sets of data (for first and for tenth flower) agree in exhibiting this maximum for the subxerophyte group, as far as the data go. As to *seed viability*, the maximum is shown for the subxerophyte group, with the value for the mesophyte group markedly lower and that for the amphibious group somewhat lower.

### COMPARISON OF THE FIRST AND SECOND GENERATIONS OF RANUNCULUS SCALERATUS

Since the second series of experiments (1916) was, in a sense, a repetition of the earlier one, it is now desirable to institute such comparisons as may be possible between the two series, and the present section will be devoted to this matter. The points to be brought out will be considered under the two captions, *environmental conditions* and *plant features*, the latter being presented under the three headings, *stem features*, *laminar area* and *reproductive features*.

#### ENVIRONMENTAL CONDITIONS

Both series of experiments were carried out in the same greenhouse, but they differed, as to environmental conditions, in two general ways. First, no attempt was made in either series to control, or even to measure adequately, any environmental feature excepting that of soil-moisture content, or water supply. All other conditions of the surroundings were allowed to be primarily determined naturally, although some conditions were modified from the natural state to a more or less unknown degree. It is clear on *a priori* grounds that the climatic conditions outside of the greenhouse must have differed in many ways for the two years involved. Also, it is probable that the temperature conditions (involving both maximum and minimum values and times and rates of temperature change), although partially controlled by artificial means, were not identical for the two periods; and the same may be said of the evaporation conditions, which were likewise artificially modified from their natural state, or partially controlled. The condition called light (involving a very complex system of wave-lengths and intensities of radiation) was primarily determined naturally; in both series of tests whatever light conditions were naturally furnished were modified to a considerable (but unknown) degree by the presence of the greenhouse roof,

which was uniformly dirty but which was painted for the second series, as has been said. That this modification was great for the solar radiation of clear midday is clearly indicated by the actinometric measurements, which were made in default of methods and apparatus for a more adequate kind of measurement, and which have been given (p. 219). Of course it is understood that there may be no definite relation between light values thus obtained and the corresponding degrees of effectiveness of the radiation in controlling plant activities. Another great group of environmental conditions that were unknown and uncontrolled, although perhaps modified from their natural state by certain experimental procedures, was that of the chemical conditions of the soil solution. Precautions were of course taken to make both aerial conditions and these soil conditions as nearly alike for all cultures as was possible, but just what they were at any time and just how they may have fluctuated remain unknown. Whatever may have been the qualities and intensities and duration of these unknown environmental conditions, they at least lay within the general limits indicated by the facts: that the outdoor climate was that of Minneapolis, that the greenhouse was heated and ventilated as for the ordinary growing of plants, and that a fairly productive garden soil was employed.

Secondly, the two series differed, in regard to the environmental conditions effective upon all culture groups, in four particulars. (1) The soil-moisture contents of the pots of the subxerophyte group were more strictly maintained in the second series than in the first—due to the employment of auto-irrigation. (2) The addition of white sand to the garden soil for the second series altered the moisture contents of all groups from their corresponding values for the earlier series, although their general relations to one another remained the same. The moisture content of the submerged soil (complete saturation) was 57.5 per cent. for the first year and 47.6 per cent. for the second, these values being calculated on the basis of the *weight* of the *dry soil*. The water-retaining capacity, with the Hilgard 1-cm. column, was 46.7 cc. and 40.4 cc., respectively for the two soils, per 100 cc. (3) The chemical properties of the soil solution may have been somewhat different in the two series, on account of the admixture of white sand to the garden soil, though any important difference in this regard may be considered as hardly probable. (4) The conditions of radiation were different for the two series, because of the paint-shading employed for the latter. This shading reduced the light value obtained with photographic paper from 0.23 (at midday with unclouded sky) to 0.035. This surely means a greatly decreased radiation intensity, at least with respect to the shorter wave-lengths of the visible spectrum. Ultra-violet radiation was surely practically all filtered out, for both series, by the greenhouse roof.

The main point to be discussed in this section is the question as to what differences and agreements were observed between the results obtained in

the first series and those obtained in the second. As has already been suggested, the difference in general light intensity was probably the most significant difference between the two environmental complexes, but the introduction of white sand into the soil of the second series may not have been without influence upon the plant differences observed. Since the other possible or probable environmental differences between the two series are not known, they will be neglected in the present discussion.

It may be mentioned that the *internal* conditions of the seeds used for the two series of tests may possibly not have been practically alike, since the seeds for the first year's work all came from the same plant while those for the later series came from plants that had grown under conditions more or less pronouncedly different from those under which the original parent had developed. Such a supposition appears, on the whole, improbable, however, but this matter will receive more attention later.

#### PLANT FEATURES

A. *Stem features.* In the second generation the plants of the xerophyte group produced no flowering stems whatever, while a few of them did in the first generation. As is shown in table VII, where the group means for corresponding features of the amphibious, mesophyte and subxerophyte groups of the two generations are brought together, both generations agreed in showing the amphibious group as having the maximum stem height and maximum height to the first flower. For both of these features the first generation showed the subxerophyte group as having the minimum value, but the minimum for total height occurred in the mesophyte group in the second generation, and the values of height to the first flower were sensibly the same for the mesophyte and subxerophyte groups in the second generation. Furthermore, the subxerophyte value for total height closely approached the amphibious (maximum) value of this feature in the second generation. In terms of the amphibious value as unity in each case, the two series (amphibious mesophyte, subxerophyte) for each of these two features are as follows. The actual amphibious value is given in parentheses in each case.

Total height	{ First generation, 1.00 (45): 0.87: 0.64
	{ Second generation, 1.00 (37): 0.89: 0.97
Height to first flower	{ First generation, 1.00 (33): 0.85: 0.64
	{ Second generation, 1.00 (31): 0.90: 0.90

For the first generation both of these feature values were progressively smaller with progressively lower water supply, while this regular relation is upset for the second generation in regard to both features, although it still holds between the amphibious and mesophyte groups of the second generation.—As to basal diameter, the maximum value occurred in the amphibious

ious group for the first generation and in the subxerophyte group for the second generation. The minimum value of this feature occurred in the subxerophyte group of the first generation, so that this feature was here progressively smaller with progressively lower water supply; but the corresponding minimum for the second generation occurred in the mesophyte group, so that this value did not here alter in the same direction as did the water supply, from the mesophyte to the subxerophyte group. The relative series for the two generations are shown below:—

Basal diameter	{ First generation	1.00 (9.7): 0.81: 0.58
	{ Second generation	1.00 (5.2): 0.94: 1.06

Regarding all three of the remaining stem features (cortex thickness, absolute thickness of aerenchyma and relative thickness of the same), the two generations agreed in showing the maximum value as occurring for the amphibious group, while the minimum value occurred uniformly for the subxerophyte group. The two relative series, in each case, are shown below:—

Thickness of cortex	{ First generation,	1.00 (790): 0.80: 0.60
	{ Second generation,	1.00 (590): 0.80: 0.76
Thickness of aerenchyma, absolute	{ First generation,	1.00 (580): 0.76: 0.41
	{ Second generation,	1.00 (500): 0.74: 0.64
Thickness of aerenchyma, in terms of cortex thickness	{ First generation,	1.00 (0.73): 0.94: 0.68
	{ Second generation,	1.00 (0.83): 0.93: 0.87

It is clear at once that all these three features showed, for both generations, progressively smaller values with lower water supply. While the two generations practically agreed as to the relative decrease in each of these values, from the amphibious to the mesophyte group (1.00:0.80, 1.00:0.75 and 1.00:0.94, for the three features, respectively), the second generation showed less relative decrease from the mesophyte to the subxerophyte group than did the first generation, in every case. In other words, the subxerophyte (minimum) value much more nearly approached the mesophyte (medium) value, for each of these three features, in the second than in the first generation.

B. *Laminar area, root-leaves.* This value showed its maximum in the amphibious group for both generations. Its minimum occurred in the subxerophyte group of the first, and in the mesophyte group of the second generation. But in the latter case the value for the subxerophyte group was nearly as small as the minimum (mesophyte group). For the first generation this feature was progressively smaller with progressively lower water supply, but this generalization holds only between the amphibious and mesophyte groups of the second generation. These two relative series follow:—

Laminar area, root-leaves	{ First generation,	1.00 (17.7): 0.64: 0.47
	{ Second generation,	1.00 (17.8): 0.78: 0.83

C. *Reproduction features.* As to the number of achenes in the first flower to open, the maximum occurred in the amphibious group of the first generation and in the subxerophyte group of the second generation. The minimum value was in the subxerophyte group of the first, and in the mesophyte group of the second generation, but the amphibious value was practically as small as the mesophyte value in the latter case. Also, for the tenth flower to open, the number of achenes showed its maximum value in the amphibious group of the first generation and its minimum in the subxerophyte group. For the second generation, this maximum was in the subxerophyte group and the minimum was in the amphibious group. It thus appears that both of these flower features showed progressively smaller values with progressively lower water supply in the first generation, that this statement is to be *reversed* for achene number in the tenth flower in the second generation (in which case the value was progressively *greater* with progressively *lower* water supply), and that the achene number for the first flower in the second generation agreed with that for the tenth flower, in the same generation, in the occurrence of the maximum in the subxerophyte group. As to seed viability, the maximum value for the first generation was again in the amphibious group, with the minimum in the subxerophyte group, so that this feature had progressively smaller values with progressively lower water supply. But this orderly relation was again upset for the second generation, in which the maximum occurred in the subxerophyte group while the minimum was in the mesophyte group. In this case the amphibious value did not approach either the maximum or minimum. The two relative series for each of these three reproduction features follow:—

Achene number, first flower	{	First generation, <b>1.00</b> (318): 0.98: 0.61
		Second generation, 1.00 (195): 1.00: <b>1.10</b>
Achene number, tenth flower	{	First generation, <b>1.00</b> (243): 0.98: 0.63
		Second generation, 1.00 (145): 1.11: <b>1.23</b>
Seed viability	{	First generation, <b>1.00</b> (97): 0.86: 0.48
		Second generation, 1.00 (55): 0.38: <b>1.27</b>

D. *Summary.* For every one of the ten plant features shown in table VII, the first generation showed a progressively smaller value with progressively lower water supply. The second generation showed this same orderly relation for cortex thickness and for both values of aerenchyma thickness, but failed to show it for the other seven features. The second generation showed the exact reverse of this relation for number of achenes in the tenth flower (progressively *lower* water supply gave progressively *larger* values). It then appears that the conditional complex, aside from water supply, was such, in the first generation, that water supply controlled, in a direct and regular manner, all the plant features here considered, while the same complex was such, in the second generation, that water supply ex-

hibited this same direct and regular control only upon the three histological features of the stem, the control being regular but *inverse* for the number of achenes in the tenth flower.

As has been pointed out, aside from the conditions of water supply, which were fairly well controlled in both generations, the other environmental conditions (especially those of light) were different for the two generations, so that it becomes interesting to compare the value for the first with that of the second generation, for each plant feature and for each group shown in table VII. This may be readily done by employing the numerical quantities given in parentheses after each feature value in the table, these quantities being, in each case, the percentage by which the first generation value is increased or decreased to give the second generation value. Examination of these parenthetical percentages brings out the following points. (Percentages of less than 10 may be considered as practically negligible.) For the amphibious group every feature value excepting three (stem height to first flower—which gave a small decrease from first to second generation,—aerenchyma thickness in terms of cortex thickness, and laminar area of root-leaves—for which the two generations were practically alike) was much smaller for the second than for the first generation. Only one (aerenchyma thickness in terms of cortex thickness) was considerably larger (14 per cent.) for the second generation than for the first. For the mesophyte group the same general statement holds excepting that in this case not only was relative cortex thickness larger (12 per cent.) but laminar area was markedly larger (23 per cent.) for the second than for the first generation. For the subxerophyte group no feature value was markedly smaller for the second generation than for the first; each of them excepting basal stem diameter and absolute cortex thickness was pronouncedly *larger* for the second than for the first generation.

It is especially interesting to note that, for all three plant groups, aerenchyma thickness in terms of cortex thickness was considerably smaller in the first than in the second generation, and that laminar area,—although it gave practically the same value in both generations for the amphibious group,—was much larger in the second than in the first generation for the mesophyte and subxerophyte groups. Another interesting generalization emerges from a study of the percentage values given in parentheses in table VII. For all features dealt with excepting the three histological stem features and seed viability, the degree of water supply seems to have determined the influence of the change in non-water-supply conditions (from the first to the second generation) upon the plants, in a perfectly consistent manner. Aside from the exceptions just noted, in those cases where high water supply gave a decreased value for the second as compared to the first generation (*minus* percentage values in the table), medium water supply gave a less pronounced, and low water supply gave a still less pronounced decrease,

or even a *negative* decrease (which is an *increase*). For laminar area the conditional change from first to second generation resulted in an *increased* value in the second generation as compared to the first, in all cases, but this increase was progressively *greater* with progressively *lower* water supply. Consequently the generalization just stated holds also for this feature, it being remembered that, as a decrease becomes smaller it passes through a state of equality and then, with change of sign, becomes an increase.

### EXPERIMENTS AND RESULTS WITH RANUNCULUS SCCELERATUS, THIRD GENERATION (1917)

With the third generation an attempt was made to discover relations between laminar area of certain plants and the water supply of their parents. Laminar area per leaf differed greatly for the amphibious and xerophyte groups of the first generation, being respectively 17.7 and 3.1 sq. cm. This was also true for the second generation, the two corresponding values being respectively 17.8 and 2.7 sq. cm. Progeny of the amphibious group of the second generation were grown, some with maximum and others with minimum water supply, and the same was done with offspring of the xerophyte group of the first generation, no seeds having been produced by the xerophyte group of the second generation.

The plants were treated as had been those of the second generation, except that seeds were sown January 12, 1917, and transplanting occurred between February 10 and March 2. In late April, from 5 to 9 mature leaves that were representative (according to visual estimate) in regard to area were removed from each plant and their laminar areas were determined by means of photographic print-paper and planimeter, the following results being obtained. In the amphibious group, three plants with "amphibious" parents had respective mean laminar areas of 13.1, 17.0 and 18.6 sq. cm., averaging  $16.2 \pm 1.3$  sq. cm., while two plants with "xerophyte" parents had respective mean laminar areas of 14.0 and 16.7 sq. cm., averaging  $15.4 \pm 1.1$  sq. cm. It will be noted that the difference is fully covered by the probable error of either average value. In the xerophyte group, three plants with "amphibious" parents had respective mean laminar areas of 3.0, 3.7 and 5.1 sq. cm., averaging  $3.9 \pm 0.6$  sq. cm., while three other plants with "xerophyte" parents had respective mean laminar areas of 3.2, 3.6 and 5.5 sq. cm., averaging  $4.1 \pm 0.6$  sq. cm. Here the difference is also obviously negligible.

From these data it seems clear that laminar area was greatly influenced by the water supply of the plants in question but that the differences, in this feature, were not at all related to the conditions of water supply to which the parents had been subjected. Of course, if such an indirect effect of

parental environment upon offspring were to occur it would mean that the influence was transmitted through the seeds. It is clear that the source of the seed was without relation to the laminar area in these tests.

EXPERIMENTS AND RESULTS WITH RANUNCULUS  
ABORTIVUS

GENERAL CONSIDERATIONS

All of the seeds for these experiments were derived from a single vigorous wild plant. They were sown in moist soil in May, 1915, and the seedlings were potted separately in early July.

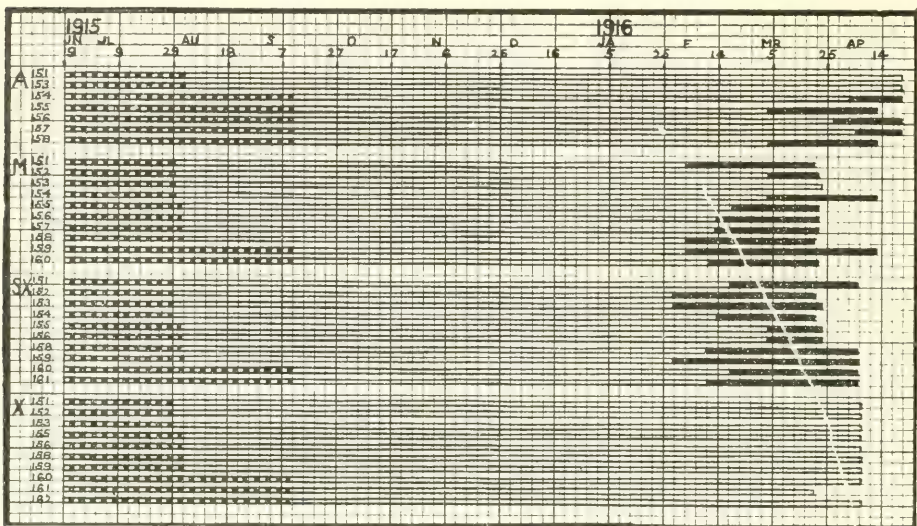


FIG. 16. Diagram showing details of the life-histories of the four groups of plants of *Ranunculus abortivus*. For description see text, p. 260.

The purely vegetative period, ending with the first opening of flowers, tended to a date between March 3 and April 4, 1916, for each plant of amphibious group, while the corresponding date was between February and March 3 for the mesophyte group, and between January 28 and March for the subxerophyte group. The xerophyte group did not show any signs of flowering when removed for examination, on April 7. All of eight submerophyte plants died within a few weeks after being potted.

The details of the life histories of the various plants of *Ranunculus abortivus* are shown graphically by the diagram of figure 16. The letters, e, s, and

numbers at the left denote, respectively, the environmental groups and individual cultures, as for the plants of *Ranunculus sceleratus* in figure 1. Here, also, similar numbers indicate similar positions in the four parallel rows of cultures (see p. 218). The horizontal bands represent, by their lengths, the

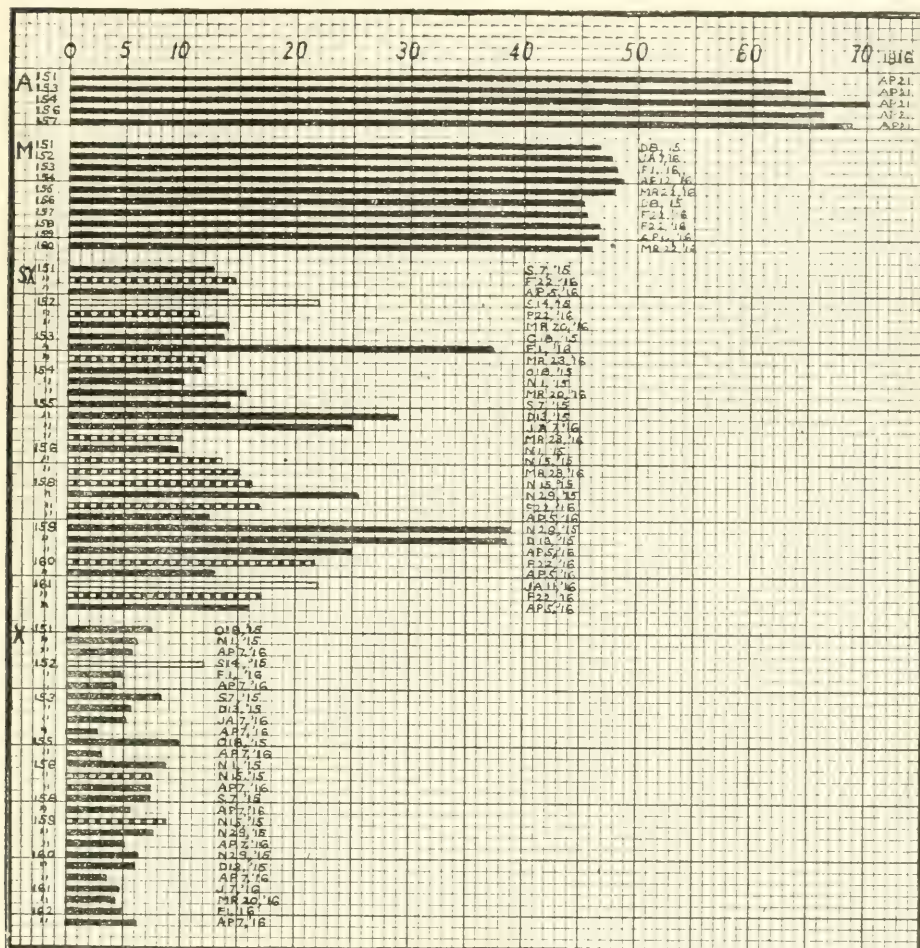


FIG. 17. Diagram showing soil-moisture contents of the various pots at several dates during the culture period of plants of *Ranunculus abortivus*. For description see text, p. 262. Compare with figure 18.

growth periods from seed. The checkered parts of these bands indicate the lengths of periods from seed sowing to the establishment of control conditions, while the solid portion of each band represents that part of the culture period following the opening of the first flower. Dates from June 19, 1915, to April 14, 1916, are shown near the upper margin of the diagram.

The data secured on the soil-moisture conditions for these four groups of cultures are shown graphically in the diagram of figure 17. Group letters and culture numbers appear at the left and the soil-moisture percentages (on the basis of dry weight) are indicated at the top. Dates of sampling are

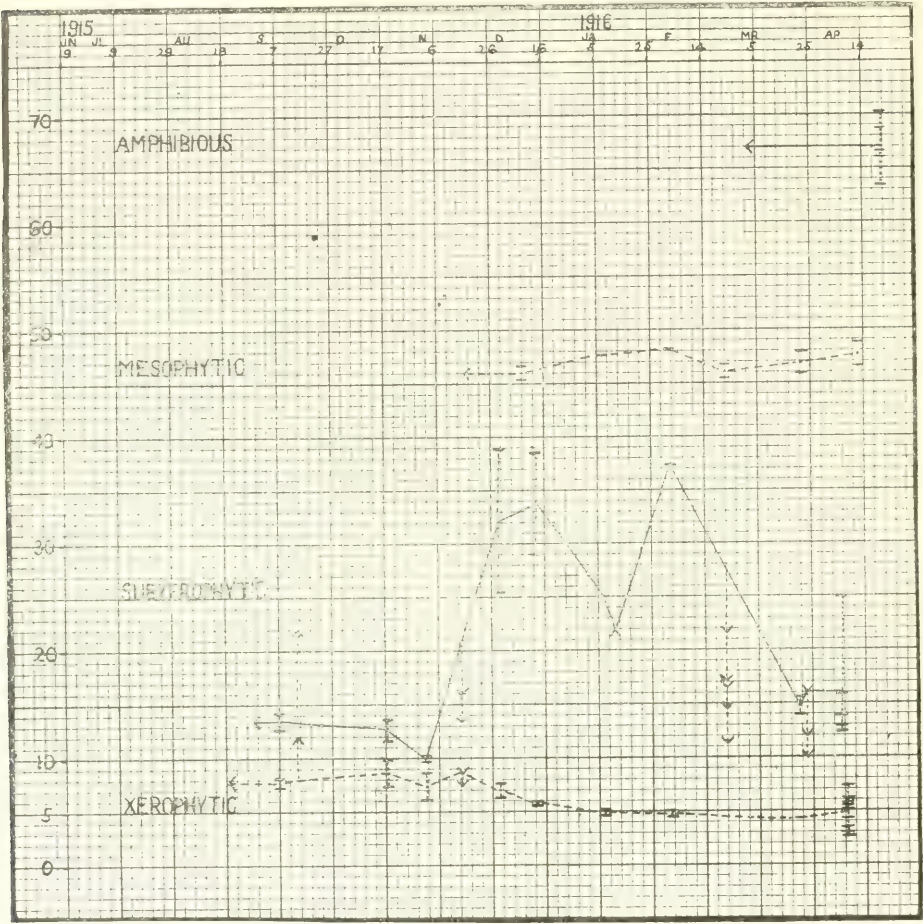


FIG. 18. Graphs showing the march of the averages of soil-moisture content for each of the four groups of plants (*Ranunculus abortivus*), as obtained by soil samples taken from the pots from time to time. For description see text, p. 263. Compare with figure 17.

represented at the right of the horizontal bands, of which each signifies by its length the percentage of moisture found at that time in that particular pot. Unshaded bands indicate moisture content of soil samples obtained just after water had been added to the pot; checkered bands represent samples

obtained just before water was added; other samples are represented by solid bands. The averages of the several data for each single date for each group of cultures are shown in the form of graphs in figure 18. These graphs represent (for each culture group) the march of the soil-moisture content as it fluctuated from time to time, as far as data for this were secured. The dates for the graphs are shown above and the percentage values are at the left. Arrows point to the left from the date of first sampling. Individual samplings (which are represented also in figure 17) are here indicated by down-

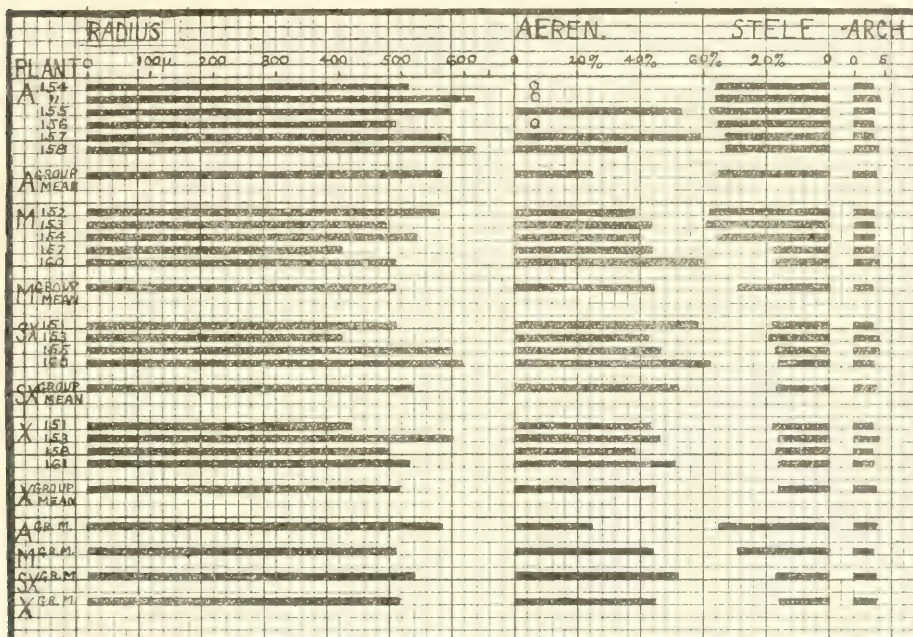


FIG. 19. Diagram showing numerical data for roots of plants of *Ranunculus abortivus*, the plants grown with four different environmental complexes. For description see text, p. 264.

ward-pointing arrowheads if they were taken just before water was added to the pots, by upward-pointing arrowheads if they were taken just after water was added to the pots, and otherwise by short horizontal lines.

The soil-moisture content for the amphibious, mesophyte, subxerophyte, and xerophyte groups, respectively, varied between 63.4 and 70.2, between 45.3 and 48.7, between 9.7 and 38.9 and between 2.6 and 9.8 per cent. The means of the extremes of these ranges are, respectively, 66.8, 47.0, 24.3 and 6.2 per cent. When these means are compared with the first, that of flooded soil, taken as the standard, they are found to have the relations, 100: 70: 36: 9.

## ROOTS

Root preparations were made and examined in the same way as for *Ranunculus sceleratus* (see p. 225), measurements being made of total root radius, stelar radius (both absolute and relative to the total radius), number of xylem strands, and thickness of aerenchyma (both absolute and relative to total radius). The data obtained from the measurements are shown diagrammatically in figure 19, which follows closely the scheme of figure 4 (see description on p. 226). In figure 19, however, each group mean is indicated twice, once directly below the set of culture values from which it is derived, and again in the lower portion of the figure. The group means, together with their probable errors, are presented in table VIII, which is self-explanatory.

TABLE VIII

*Average numerical data for roots of Ranunculus abortivus, the plants grown with four different environmental complexes*

ANATOMICAL FEATURE		AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXERO-PHYTE GROUP	XEROPHYTE GROUP
Root radius, <i>micra</i> .....		565±16	491±17	518±34	496±24
Stelar radius of root	Absolute, <i>micra</i> .....	197±6	145±24	90±3	80±3
	Relative, <i>per cent.</i> of root radius .....	35±1	29±4	17±.5	16±.4
Number of xylem strands in root .....		3.3±.1	3.2±.1	3.5±.2	3.3±.2
Thickness of root aerenchyma	Absolute, <i>micra</i> .....	140±53	214±9	270±25	226±21
	Relative, <i>per cent.</i> of root radius .....	25±9	44±3	52±4	45±2

A study of the average values shown in table VIII brings out the following generalizations. The root radius was somewhat greater in the amphibious group than in the subxerophyte group and markedly greater in the amphibious than in the mesophyte and xerophyte groups. This feature showed no regular relation to water supply. The absolute radius of the stele was greatest for the amphibious and least for the xerophyte group, showing progressively smaller values with progressively lower water supply. This generalization is also true for the stelar radius relative to that of the root, although the differences between groups were not quite so distinct for these relative values. The number of xylem strands was about the same for all groups and no relation between this feature and water supply is apparent. The layer of aerenchyma was thickest for the subxerophyte group, somewhat thinner for the xerophyte group, markedly thinner for the mesophyte group, and thinnest for the amphibious group. For the first three groups this feature

shows an inverse relation to water supply, the value being progressively larger with progressively lower water supply. The relative thickness of aerenchyma (per cent. of root radius) was greatest for the subxerophyte and least for the amphibious group, being of medium values for the mesophyte and xerophyte groups, so that an inverse relation to water supply is strongly suggested.

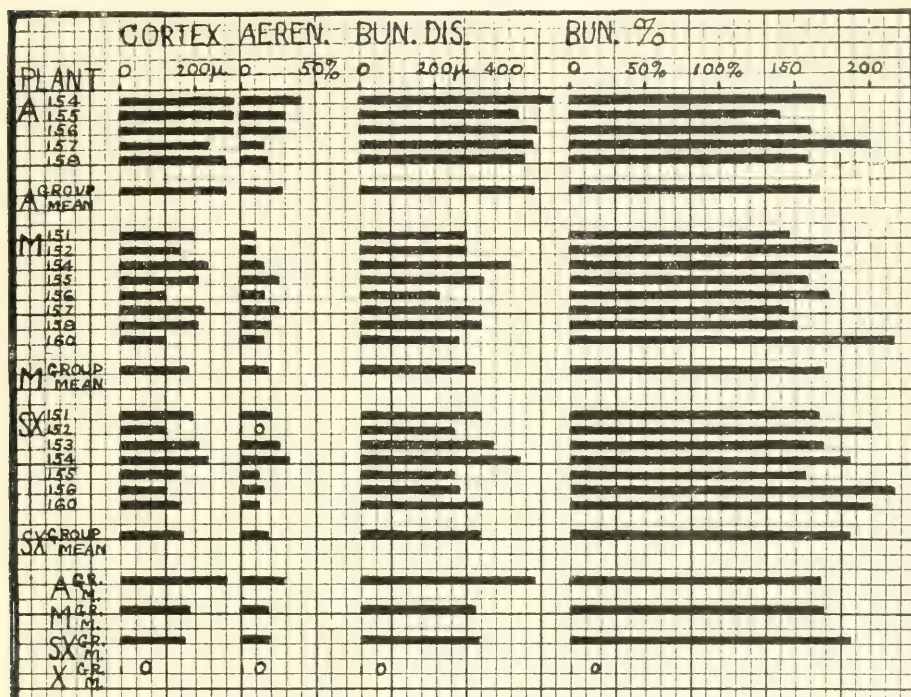


FIG. 20. Diagram showing numerical data for stems of plants of *Ranunculus abortivus*, the plants grown with four different environmental complexes. For description see text p. 266.

#### STEMS

As has been pointed out, no flower-bearing stems were produced by plants of the xerophyte group. In the plants of the other groups their number varied, the stem that developed from the terminal bud always being larger than the one or more stems usually developing from lateral buds in the axils of the root-leaves. These larger terminal stems were used for microscopic examination, but they were not measured in regard to total diameter and height, as were those of the plants of *Ranunculus sceleratus*, because these gross features were markedly smaller when there was more development of lateral stems. On the other hand, the microscopic features that were meas-

ured showed no differences to correspond with the development of lateral stems. The microscopic measurements were made as for the plants of *Ranunculus sceleratus* (see p. 227), for thickness of cortex, thickness of aerenchyma (absolute and relative to thickness of cortex), and bundle interval (absolute and relative to cortex thickness).

Most of the results of the various individual measurements, and also the group means, are shown in the diagram of figure 20, where the same scheme is used as for figure 5 (see description on p. 228) except that each group mean is given twice, once directly below the set of culture values for which it is the average, and again in the lower part of the figure. The group means and their probable errors are presented in table IX, which is self-explanatory.

From the group means shown in table IX and figure 20, it is obvious that the amphibious group was foremost in regard to each feature measured

TABLE IX

*Average numerical data for stems of Ranunculus abortivus, the plants grown with four different environmental complexes*

ANATOMICAL FEATURE		AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXERO-PHYTE GROUP
Thickness of stem cortex, <i>mica</i> . . . . .		282±10	183±11	179±15
Thickness of stem aerenchyma	Absolute, <i>mica</i> . . . . .	77±11	32±5	30±7
	Relative, per cent. of cortex thickness . . . . .	27±4	17±2	18±3
Bundle interval of stem	Absolute, <i>mica</i> . . . . .	460±10	300±10	310±17
	Relative, per cent. of cortex thickness . . . . .	164±7	167±6	182±6

excepting the relative bundle interval, while the other two groups that had stems (mesophyte and subxerophyte) were markedly lower and about alike in each case. It may be stated that the first two groups show a direct relation between each of these four values and water supply. The relative values of the bundle interval appear, on the other hand, to show an inverse relation to water supply, the minimum occurring in the amphibious group and the maximum in the subxerophyte group.

#### LEAVES

Root-leaves alone were examined, in regard to two petiolar and eight laminar characters, the same foliar features as were studied for *Ranunculus sceleratus* (see p. 231). These leaves were unlobed until a few weeks before the production of the flowering stem, and then the new ones formed were progressively more deeply lobed. Not more than three leaves from each plant were

examined. On February 1, 1916, a mature, unlobed leaf of average size (by visual estimate) was removed from each plant. In addition, the lobed leaf first to appear and the one last to appear (i.e., the one next to the terminal flowering stem) were also removed when mature. The same method was used for making the examination and measurements as was used in the case of *Ranunculus sceleratus* (see p. 232).

The various leaf data obtained are mostly presented by the diagrams of figures 21-24, where the same general scheme is followed that was followed for figures 8-12. Group letters, plant numbers, the dates of removal of leaves, and the leaf numbers, are noted at the left of each diagram. The

TABLE X

*Average numerical data for leaves of Ranunculus abortivus, the plants grown with four different environmental complexes*

ANATOMICAL FEATURE		AMPHIBIOUS GROUP	MESOPHYTE GROUP	SUBXERO-PHYTE GROUP	XEROPHYTE GROUP		
Petioles	No. of bundles .....	11.0±.1	8.5±.4	7.5±.4	6.0±.3		
	Width, mm.....	3.4±.2	2.8±.1	2.3±.1	2.1±.1		
Laminar area, sq. cm.....		20.4±.6	12.0±.8	14.0±1.1	4.9±.2		
Stomatal frequency, per 0.1 sq. mm.	Lower surface...	9.2±.2	7.4±.3	8.4±.4	11.8±.5		
	Upper surface..	5.8±.2	5.0±.2	4.6±.4	8.2±.3		
Chloren- chyma	Entire... }	No. of layers.....	6.4±.1	6.4±.1	6.5±.1	6.4±.1	
		Thickness, micra..	162±4	149±3	151±3	158±3	
	Palisade	Thickness <sup>a</sup>	No. of layers.....	1.6±.1	1.6±.1	1.6±.1	2.0±.01
			Absolute, micra	44±.7	39±.6	39±.6	37±.6
			Relative, per cent. of entire chlorenchyma	27±1	26±.4	26±1	23±1

<sup>a</sup> Thickness here concerns only the external layer of palisade; see p. 231.

average for the root-leaves of each plant is given in the lower part of the diagram, as a plant average (e.g., "A151 ave." in fig. 21), and such plant means are averaged in their turn to give the group mean. The various features are designated and represented as for figures 8-13 (see description on p. 233).

The group means from the data given in the diagrams of figures 21-24 are shown in table X which is self-explanatory. It should be stated that, although occasionally only one or two leaves were secured from a plant and although the third or most-lobed leaf usually had the largest laminar area, nevertheless group means derived from the averages of the three types of leaves were practically the same as those derived from the plant averages.

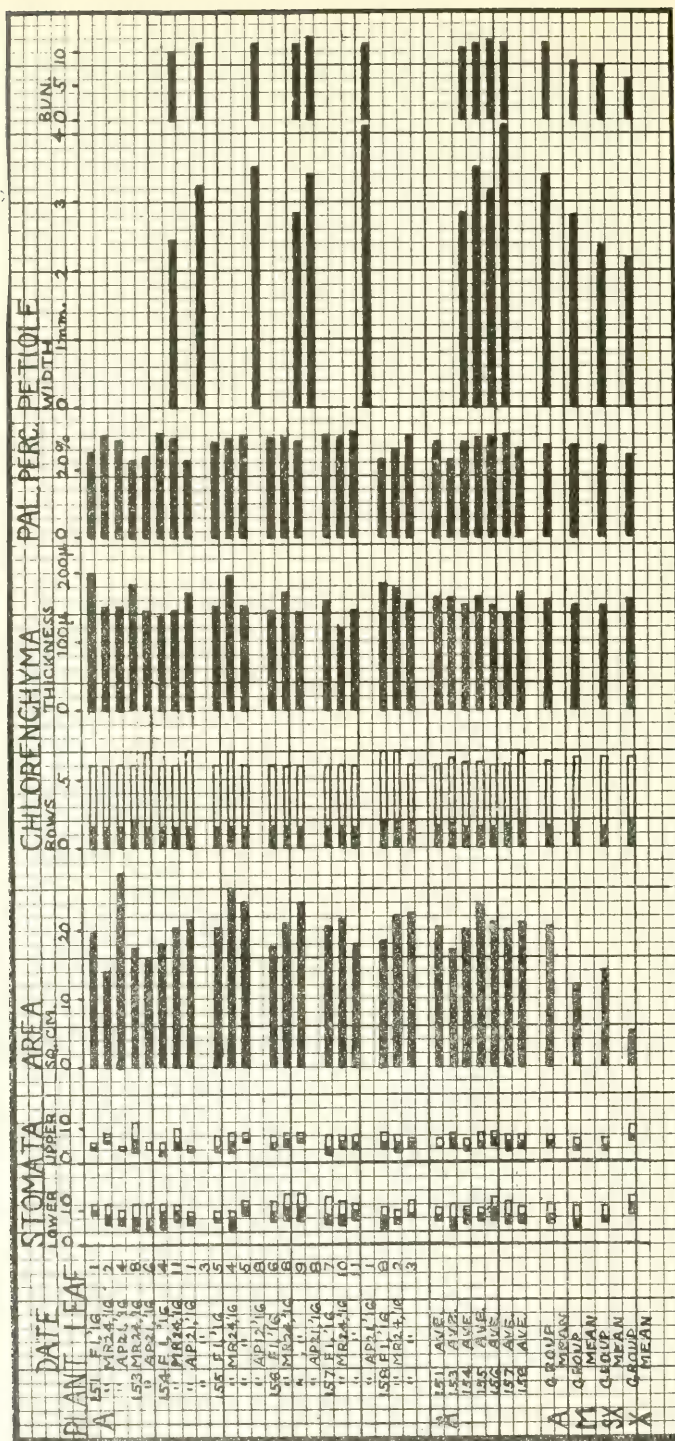
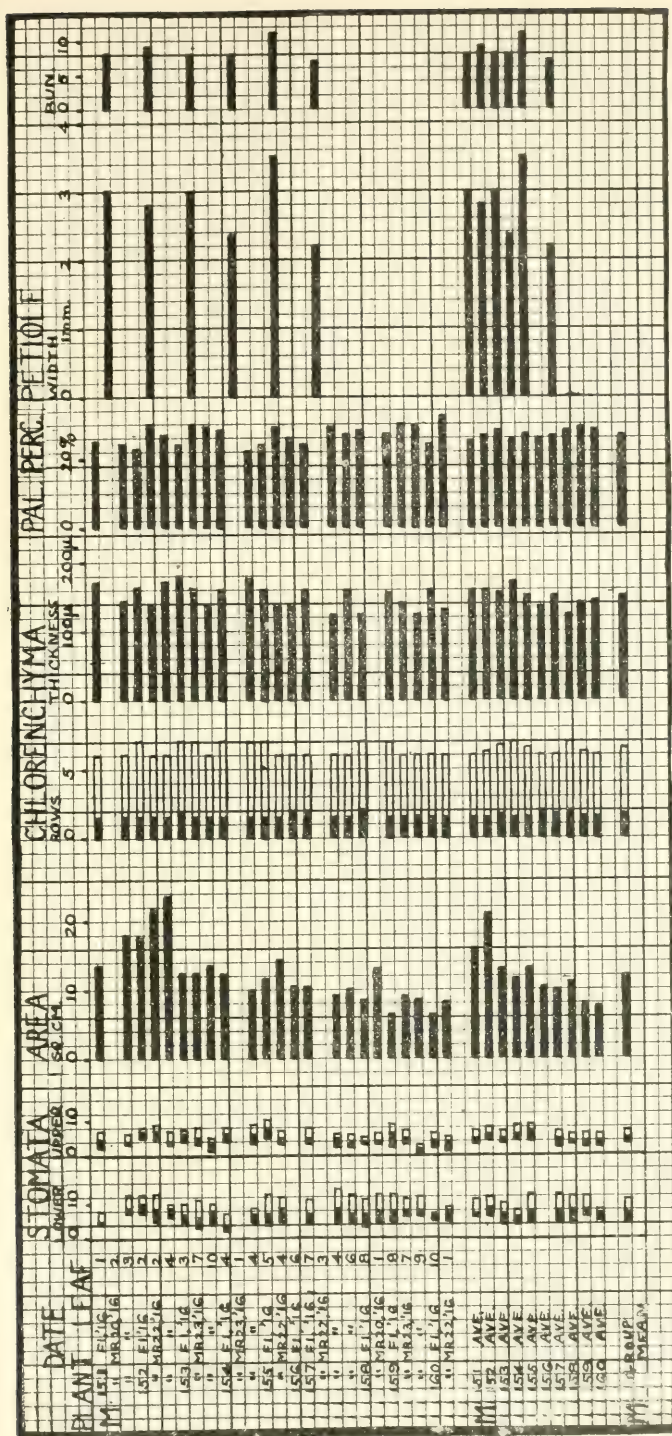


FIG. 21. Diagram showing numerical data for leaf features of the *amphibious* group of cultures of plants of *Ranunculus abortivus*. For description see text, p. 267. Compare with figures 22-24.



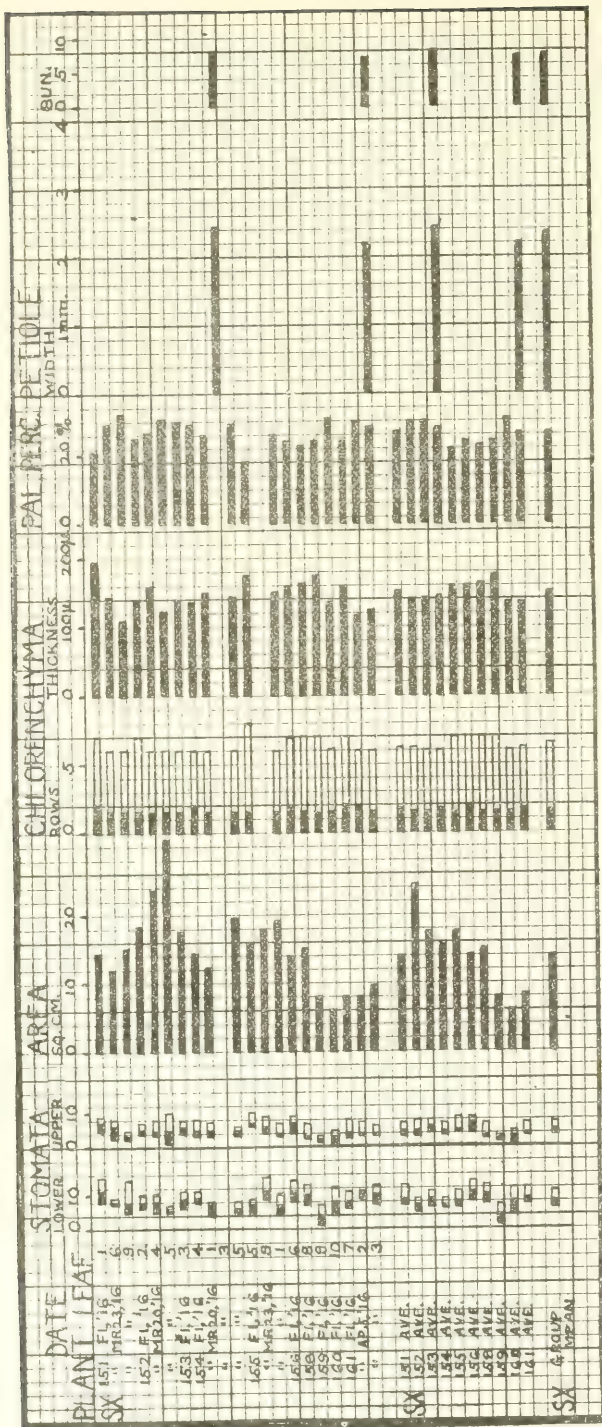


FIG. 23. Diagram showing numerical data for leaf features of the *subserophylla* group of cultures of plants of *Rauvolfia abortiva*. For description see text, p. 267. Compare with figures 21, 22 and 24.

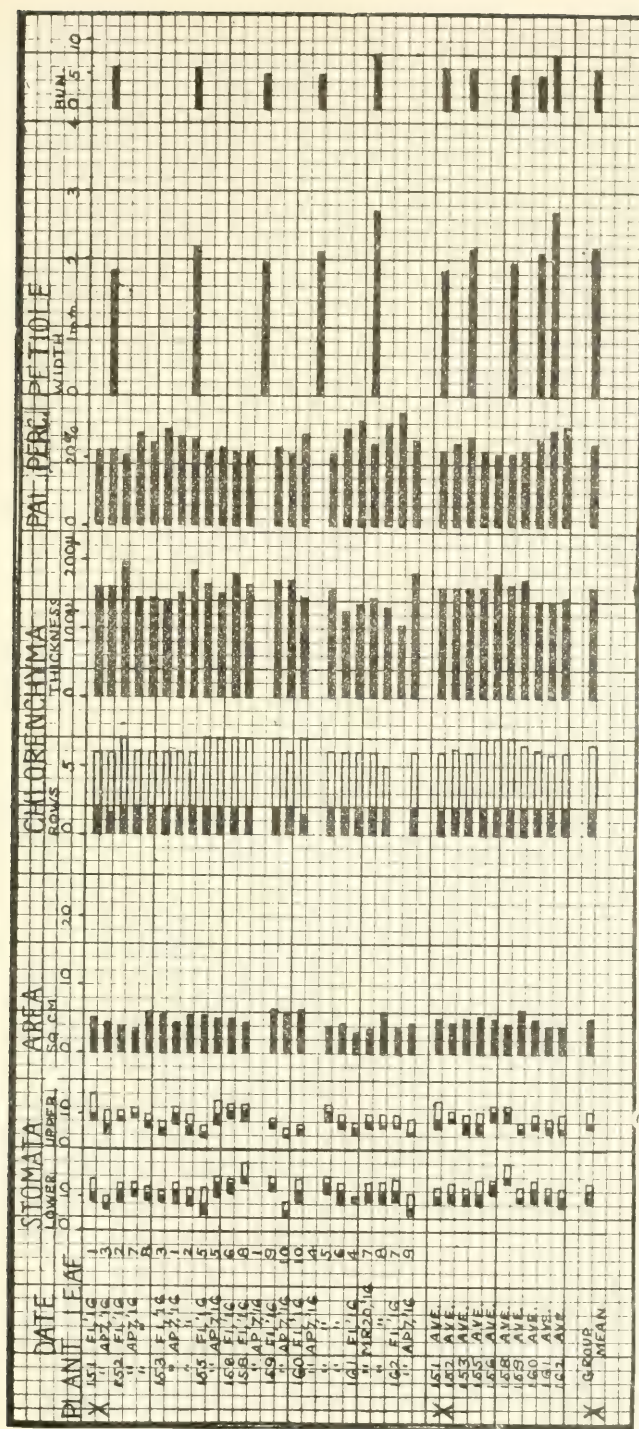


FIG. 24. Diagram showing numerical data for leaf features of the xerophyte group of cultures of plants of *Ranunculus abortivus*. For description see text, p. 267. Compare with figures 21-23.

Examination of table X and of the group means at the base of figure 21 brings out the following generalizations regarding the various anatomical features of the root-leaves. The *number of petiolar bundles* was greatest for the amphibious group and progressively smaller with progressively lower water supply, and the same was true regarding the *width of the petiole*, although not so distinctly so. *Laminar area* was greatest for the amphibious group, of median value for the mesophyte and subxerophyte groups (the latter having somewhat the greater area) and was least for the xerophyte group. *Stomatal frequency for the lower leaf surface* was greatest for the xerophyte group, next highest for the amphibious group, and least for the mesophyte group. The same generalization is true of *stomatal frequency for the upper leaf surface*, except that this value was about the same for the mesophyte and subxerophyte groups. The *number of layers of chlorenchyma cells* was about alike for all groups. The *number of layers of palisade cells* was the same for all except the xerophyte group, for which it was greatest. The *thickness of the chlorenchyma* was somewhat greater for the amphibious and xerophyte groups than for the mesophyte and subxerophyte groups. The *absolute thickness of palisade* (external layer only) was greatest for the amphibious group, least for the xerophyte group, and of median and similar values for the mesophyte and subxerophyte groups. The *relative thickness of palisade* (in percentage of the entire chlorenchyma thickness) was about the same for all except the xerophyte group, for which it was least.

It consequently appears that the two leaf features, number of bundles in the petiole and petiolar width, are the only ones of those here considered that showed a direct and consistent relation to the water supply of the plants; for these features progressively smaller values occur with progressively lower water supply. This same relation is, however, partially indicated for three other of these leaf features, laminar area, absolute palisade thickness and relative palisade thickness; in these three cases the mesophyte and subxerophyte groups showed equal, or nearly equal, values, which were intermediate between the maximum (amphibious group) and minimum (xerophyte group). In no case was an inverse and consistent relation shown, but this is partially indicated for two features, stomatal frequency for the lower surface and the number of palisade layers. The remaining three features (stomatal frequency for the upper surface, number of chlorenchyma layers and thickness of chlorenchyma) showed no apparent relation at all in this connection.

COMPARISON OF *R. SCCLERATUS* AND *R. ABORTIVUS*

A comparison between the two species of *Ranunculus* here studied may be instituted, with respect to agreements and disagreements as to their relation to water supply. In order to bring this complicated series of relations together in a way to facilitate comparisons, all the feature values obtained for both species are presented in table XI, where each one is stated in terms of the corresponding value for the amphibious group taken as unity. The arrangement is similar to that resorted to in comparing the relations shown by the two generations of *R. sccleratus*. The actual value for the amphibious group is given in parentheses, in each case. Where four relative values are given in the series the last is for the xerophyte group; where only three appear the last is for the subxerophyte group. The corresponding series of relative values derived from the first generation of *R. sccleratus* are the ones presented in table XI; it will be remembered that the experimental series representing the first generation of *R. sccleratus* was roughly coextensive with the experiments with *R. abortivus* (summer and fall of 1915); it would be useless to attempt to compare the results from the second generation of *R. sccleratus* with those obtained with *R. abortivus*, since the environmental complex, aside from water supply, was definitely different for these two experimental series.

In table XI, at the right of each series of relative values, for each plant, is placed a conventional sign to indicate the nature of the apparent relation holding between the feature value and water supply, this relation being judged from the group means (given in tables II – IV) with their probable errors. A *plus* sign (+) indicates a clear case of direct relation; with progressively *lower* water supply the value is progressively *smaller*. The same sign followed by an interrogation point (+?) indicates that a direct relation is suggested or partially indicated, but is not clear throughout the series. A *minus* sign (–) indicates a clear case of inverse relation; with progressively *lower* water supply the value is progressively *larger*. This sign followed by an interrogation point (–?) denotes a suggested or partially indicated inverse relation. A *zero* (0) denotes no apparent and consistent relation to water supply, while the same sign followed by a sign of equality (0=) indicates that all plant groups have approximately equal values.

No feature exhibits a consistent *direct* relation to water supply (+) for both species. Considering that a direct relation is shown whether it be consistent or not (+ or +?), the following features may be said to belong in the category of those for which a direct relation to water supply is at least suggested in both species:—(1) Thickness of stem cortex. (2) Thickness of stem aerenchyma, both absolute and relative to cortex thickness. (3) Stem bundle interval, absolute. (4) Number of bundles in root-leaf petiole. (5) Petiolar width of root-leaves. (6) Laminar area of root-leaves.

TABLE XI

Relative plant feature values, each in terms of corresponding value for *R. abortivus* group, for *R. abortivus* and *R. sceleratus* (first generation). For explanation and meanings of conventional signs see text

	R. ABORTIVUS	RELATION TO WATER SUPPLY	R. SCLERATUS	RELATION TO WATER SUPPLY
Root radius.....	1.00 (.565): .87: .92: .88	0	1.00 (.655): 1.03: .88: .57	+?
Stelar radius, root, absolute.....	1.00 (.197): .74: .46: .41	+	1.00 (.99): .96: 1.41: .67	0
Do., relative.....	1.00 (.35): .83: .49: .46	+	1.00 (.15): .93: 1.60: 1.13	0
No. xylem strands, root.....	1.00 (.3.3): .97: 1.06: 1.00	0=	1.00 (.3.3): 1.06: 1.21: .97	0
Root aerenchyma thickness, absolute.....	1.00 (.140): 1.53: 1.93: 1.60	-?	1.00 (.438): 1.00: .62: .39	+?
Do., relative.....	1.00 (.25): 1.76: 2.08: 1.80	-?	1.00 (.67): .97: .70: .67	+?
Stem cortex, thickness.....	1.00 (.282): .65: .60	+?	1.00 (.790): .80: .60	+
Stem aerenchyma, thickness, absolute.....	1.00 (.77): .42: .39	+?	1.00 (.580): .76: .41	+
Do., relative.....	1.00 (.27): .63: .67	+?	1.00 (.73): .94: .68	+
Stem bundle interval, absolute.....	1.00 (.460): .65: .67	+?	1.00 (.580): .85: .66	+?
Do., relative to cortex thickness.....	1.00 (.164): 1.04: 1.11	-	1.00 (.73): 1.07: 1.11	-
No. bundles in petiole.....	1.00 (.11.0): .77: .68: .55	+	1.00 (.9.0): .86: .92: .41	+?
Petiole width.....	1.00 (.3.4): .82: .68: .62	+	1.00 (.2.6): 1.04: .73: .42	+?
Laminar area.....	1.00 (.20.4): .59: .69: .24	+?	1.00 (.17.7): .64: .47: .18	+
Stomatal frequency, lower surface.....	1.00 (.9.2): .80: .91: 1.28	-?	1.00 (.2.6): 1.38: 1.38: 1.04	0
Do., upper surface.....	1.00 (.5.8): .86: .79: 1.41	0	1.00 (.7.4): 1.08: .85: .92	0
No. layers chlorenchyma.....	1.00 (.6.4): 1.00: 1.02: 1.00	0=	1.00 (.7.2): .96: .92: .92	+?
Thickness of chlorenchyma.....	1.00 (.162): .92: .93: .98	0=	1.00 (.201): .97: .95: .60	+?
No. layers palisade.....	1.00 (.1.6): 1.00: 1.00: 1.25	-?	1.00 (.1.7): .94: .88: .94	0=
Thickness of palisade, absolute.....	1.00 (.44): .89: .89: .84	+?	1.00 (.64): .98: .98: .55	0
Do., relative.....	1.00 (.27): .96: .96: .85	+?	1.00 (.32): 1.00: 1.03: .91	0=

The two species agree in showing a consistent *inverse* relation (—) to water supply only with respect to the single feature, stem bundle interval relative to cortex thickness, while an inverse relation (—?) is not suggested for both species in regard to any feature.

With respect to the following features the two species agree in showing no apparent relation (0) to water supply:—(1) Number of xylem strands in root. (2) Stomatal frequency, upper surface of root-leaves.

The two species are indicated as in disagreement, regarding their relation to water supply, for the following features:—(1) Root radius. (2) Stelar radius of root, both absolute and relative to root radius. (3) Thickness of root aerenchyma, both absolute and relative to root radius. (4) Stomatal frequency of lower surface of root-leaves. (5) Number of layers of chlorenchyma. (6) Thickness of root-leaf chlorenchyma. (7) Number of layers of root-leaf palisade. (8) Thickness of root-leaf palisade, both absolute and relative to chlorenchyma thickness.

Of course no great weight should be attached to the results thus brought out by the comparison of the two species, but they may be of interest in connection with future studies along this general line.

### CONCLUSION

A summary of the main results obtained is given in the Abstract at the beginning of this paper, and need not be repeated here. A few remarks may be added, however.

From the results obtained in the first generation of *R. sceleratus* it appears probable that the differences between Glück's [1911] six forms of this species (see p. 215 of the present paper) and those may have been related to environmental conditions, especially to water supply. The occurrence of "normal" and "dwarf" forms of this species side by side in the field, as noted by Glück, may have been due to competition resulting in a deficiency in the water supply for the "dwarf" individuals. As has been pointed out, the writer has grown progeny of the same plant with different degrees of water supply and has thus produced "dwarf," "normal" and other forms, in adjacent pots, and then has found the offspring of "dwarf" and "normal" forms to be alike when treated alike, either with wet or dry soil.

From the comparison between the first and second generation of *R. sceleratus* it is suggested that the fact that F. E. Clements [1905] obtained more different forms of this species than are here reported (nine, as has been mentioned on p. 215) may be due to different non-water-supply conditions from those employed in the present study, his work having been done at Lincoln while the present work was carried out at Minneapolis. Clements used a larger soil mass for each plant and the light conditions were surely different in his experiments, since the sun's seasonal altitude is greater for Lincoln than

for Minneapolis and since the greenhouse at the latter station was shaded either by much dirt or by paint on the glass (see p. 219), such artificial shade having been absent in Clements' experimentation. In the work here reported, most of the conditions were not under absolute control, while, on the other hand, the greenhouse roof made it impossible to consider these plants as being under natural conditions. When such experimentation as the present is to be continued, or when similar lines are to be attacked, it is highly desirable that more attention be given to methods for the artificial control of environmental conditions or for the adequate measurement of conditions that are not artificially controlled, than has yet been done in this sort of work.

### LITERATURE CITED

Numbers in brackets throughout the preceding pages refer to the year of publication as given in this list.

- BONDOIS, G., Contributions à l'étude de l'influence du milieu aquatique sur les racines des arbres. *Ann. Sci. Nat. Bot.* **IX**, 13: 1-24. 1913.
- BRIGGS, L. J., AND H. L. SHANTZ, The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agric., Bur. Plant. Ind., Bull. 230. 1912.
- , The water requirement of plants. I. Investigations in the great plains in 1910 and 1911. U. S. Dept. Agric., Bur. Plant. Ind., Bull. 284. 1913.
- , The water requirement of plants. II. A review of the literature. U. S. Dept. Agric., Bur. Plant. Ind., Bull. 285. 1913.
- BURNS, G. P., Heterophylly in *Proserpinaca palustris* L. *Ann. Bot.* **18**: 579-587. 1904.
- CASTLE, W. E., Selection, sugar-beets and thrips. *Amer. Nat.* **49**: 121-122. 1915.
- CHAMBERLAIN, C. J., Methods in plant histology. xi + 314 p. Chicago, 1915.
- CLEMENTS, EDITH S., The relation of leaf structure to physical factors. *Trans. Amer. Microsc. Soc.* **1905**: 19-102. 1905.
- CLEMENTS, F. E., Research methods in ecology. xvii + 334 p. Lincoln, 1905.
- GLÜCK, H., Biologische und morphologische Untersuchungen über Wasser- und Sumpfgewächse. 3 vols. Jena, 1905-1911.
- HAWKINS, L. A., The porous clay cup for the automatic watering of plants. *Plant World* **13**: 220-227. 1910.
- HILGARD, E. W., Soils, their formation, properties, composition, and relations to climate and plant growth in the humid and arid regions. xxx + 593 p. New York and London, 1910.
- HOLMES, F. S., Moisture equilibrium in pots of soil equipped with auto-irrigators. *Johns Hopkins Univ. Circ.* March, 1917, p. 208-210.
- KIESSELBACH, T. A., Transpiration as a factor in crop production. *Nebraska Agric. Exper. Sta. Research Bull.* **6**. 1916.
- LIVINGSTON, B. E., A method for controlling plant moisture. *Plant World* **11**: 39-40. 1908.
- LIVINGSTON, B. E., AND L. A. HAWKINS, The water-relation between plants and soil. *Carnegie Inst. Wash. Pub.* **204**: 1-48. 1915.
- LYON, T. L., AND E. O. FIPPIN, The principles of soil management. xxxiii + 531 p. New York, 1909.
- MACDOUGAL, D. T., The determinative action of environic factors upon *Neobeckia aquatica* Greene. *Flora* **106**: 264-280. 1914.
- MCCALLUM, W. B., On the nature of the stimulus causing the change of form and structure in *Proserpinaca palustris*. *Bot. Gaz.* **34**: 93-103. 1902.
- POOL, R. J., A study of the vegetation of the sandhills of Nebraska. *Minnesota Bot. Studies* **4**: 189-312. 1914.
- SHAW, H. B., Thrips as pollinators of beet flowers. U. S. Dept. Agric., Bull. 104. 1914.
- YUNCKER, T. G., A study of the relation of soil moisture to transpiration and photosynthesis in the corn plant. *Plant World* **19**: 151-161. 1916.
- ZELENY, A., AND H. A. ERIKSON, A manual of physical measurements. vii + xv + 147 p. New York, 1912.

# THE QUANTITATIVE DETERMINATION OF PHOTOSYNTHETIC ACTIVITY IN PLANTS

FRANCES LOUISE LONG

## ABSTRACT<sup>1</sup>

This paper reports results of an endeavor to find a satisfactory chemical method of determining photosynthetic activity, in the hope of being able to apply this in measuring the activities of various species in the same habitat, and of the same species in various habitats. Various chemical methods were tried. The "Munson-Walker" method, modified to secure uniform heating of the solutions and complete collections of the copper oxide precipitated, was found to be the most reliable for the determination of small quantities of carbohydrates.

In order to test this method as to its ecological use, determinations of photosynthetic activity were made for plants under a variety of habitat conditions. Although time was lacking to try these out adequately, the results obtained are suggestive and promising. Further work along this line is in progress.

An indication of the general behavior of plants in the formation of carbohydrate was obtained by a series of determinations for healthy leaves of *Phaseolus*, made at different hours of the day. The amount of carbohydrate shown in the leaves gradually increased from early morning until the hour following midday. Of the total photosynthate, 13 per cent. was made during that hour. A relatively high activity continued until four in the afternoon when a rapid decrease began. The hours of greatest activity correspond to the hours of greatest light intensity, 80 per cent. and above.

Similar photosynthetic behavior was noted in *Taraxacum* during the morning hours, but the maximum was reached at two o'clock in the afternoon. After this time, there was a decrease in the total photosynthetic activity each hour, which probably indicates that translocation and respiration were taking place more rapidly than manufacture.

With reference to the relation of leaf position to photosynthesis no correlation was found in the case of *Helianthus*. The leaves at the top of the stem showed less carbohydrate than the others, but this may be accounted for by supposing that such young leaves use the carbohydrates for growth as rapidly as these are made.

---

<sup>1</sup> The manuscript of this paper was received December 10, 1918. This abstract was preprinted, without change, from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 2, no. 6, August, 1919.

A comparison of photosynthetic activity in normal and in inverted leaves of *Helianthus* showed a higher carbohydrate content in the inverted leaves. A possible explanation is seen in the fact that the sponge tissue may become more efficient in the inverted leaves while the efficiency of the palisade may be greatly reduced. It may be that the larger carbohydrate content of inverted leaves is largely due to the twisting of the petiole, retarding translocation.

A comparison of the different leaves of the *Taraxacum* rosette showed that those next to the earth made less than half as much photosynthate as did the leaves on the top. The leaves in the middle portion were only slightly more active than the lowest.

When the light value was reduced to 10 per cent. the photosynthetic activity of *Phaseolus* was reduced to 6 per cent. A reduction of the light intensity to 0.3 per cent. decreased the photosynthetic activity to 2 per cent.

The effect of submergence under water was to decrease the photosynthate in proportion to the depth of submergence for the first few centimeters below the surface.

In the case of stamens, a determination of the average carbohydrate content for two days, morning and evening, showed that about 8.1 mg. of dextrose per gram of dry material was made daily. This may indicate that temporary structures, as stamen filaments, are utilized to help supply the unusual demands that come at the time of flowering.

A study of the relation of transpiration to photosynthetic activity in *Equisetum fluviale*, *Helianthus annuus* and *Phaseolus vulgaris* showed that relative transpiration and photosynthetic activity were about inversely proportional. The transpiration rate (grams per square centimeter) for *Equisetum* was 0.1520; for *Helianthus*, 0.0980; for *Phaseolus* 0.0650. The photosynthate shown (milligrams per square centimeter) for *Equisetum* was 0.1500; for *Helianthus* 0.2802; for *Phaseolus* 0.4491.

Changes in the quality of light made a marked difference in the photosynthetic activity. Blue light was found to be least active for photosynthate production, and red the most active.

Rusts and mildews interfered with photosynthetic activity in all cases tested. In *Avena* affected by *Puccinia coronata*, the rust (when well developed but not erumpent) reduced the photosynthetic activity to 72 per cent., and in the erumpent stage to 48 per cent. A comparison of the infected with the non-infected regions showed that the former contained only 21 per cent. as much photosynthate as did the latter.

*Puccinia graminis* on *Triticum* reduced the photosynthate to 50 per cent. in the stage before the pustules become brown, and in the erumpent stage, to 39 per cent. For equal areas, the infected region showed 8 per cent. as much photosynthate as was made in the other parts of the leaf.

*Uromyces* on *Arisaema* decreased the photosynthate to 78 per cent. when the sori were well developed, but still white. When the spores became yellow and the sori were crumpled, a decrease to 40 per cent. in carbohydrate production was shown.

Leaves of *Aquilegia* with mycelium of *Erysiphe* covering half the surface showed a reduction in photosynthetic activity to 95 per cent. Where the whole leaf was infected the reduction was to 55 per cent.

Animal parasites reduced the apparent activity of the leaves of the host, probably by abstracting food and to a small extent by reducing the quantity of light received. In leaves of *Phaseolus* partly infected with *Tetranychus telarius* (red spider), the amount of dextrose produced was reduced to 37 per cent. In badly infected plants there was a reduction to 40 per cent. in the amount of photosynthate produced.

*Aphis* reduced the carbohydrate present in the *Phaseolus* leaves to 96 per cent.

## INTRODUCTION

During the past decade much quantitative work has been done on the water relations of plants. As a consequence, we now have an excellent working knowledge of the water efficiency of the majority of the common field and garden crops. This has been expressed in such terms as the wilting coefficient, chresard, relative transpiration and water requirement. In the other fundamental relation, that of light, very little has yet been done beyond the measurement of light values in different habitats and communities. It is clear that the total efficiency of any species depends upon its light requirements as well as upon its water requirements. While growth has often been used as a measure of light efficiency, it is evident that this process is directly related to water as well. As a result, it is necessary to turn to the process of photosynthesis for an exact determination of the light requirement. Fortunately, the end product of photosynthesis, the photosynthate, lends itself to exact quantitative work. The primary objective of the present investigation was to develop a rapid and fairly precise method of measuring photosynthetic efficiency, and its variation under different conditions. A preliminary attack has also been made upon the effect of parasitic plants and animals upon photosynthesis in the host. While the present study affords a mere beginning in this vast field, it is felt that the methods developed and results obtained may blaze the way toward further study that may finally result in an adequate knowledge of the light requirements of plants.

Kind acknowledgment is made of the advice and criticism given by Dr. Frederick E. Clements, who suggested the problem. The writer is also indebted to Dr. E. M. Freeman, Dr. R. A. Gortner, Dr. W. H. Hunter and Dr. E. P. Harding for helpful suggestions in carrying on the work.

## METHODS EMPLOYED

## THE LIGHT FACTOR

## MEASUREMENTS OF LIGHT INTENSITY

*The Stop-Watch Photometer.* A very satisfactory instrument for making ordinary light readings is the stop-watch photometer devised by Clements, and manufactured by the C. H. Stoelting Co. An exposure to light is made by quickly opening the shutter, which operation also starts the stop-watch. The watch stops as the shutter is closed at the end of the exposure. After exposure, the wheel on which the "solio" paper strip is mounted is rotated  $\frac{1}{25}$  of the circumference, to the point marked "exposure 2." This places an unexposed square of sensitive paper in position for the next exposure. All light readings are evaluated by comparing them with a standard. This standard is made by a series of exposures to full noon sun, out-doors, for periods varying from 1 to 10 seconds. It is better to make two consecutive series of exposures for the standard. The paper strip bearing the records to be determined are placed between the two series of standard readings as they lie vertically. The strip is moved up and down until the place is found where the tones of the two standards and that of the other exposure match. If the standard is the result of a 10-second exposure and the record which corresponds in color requires 100 seconds, then the light intensity for the latter is considered as  $\frac{1}{10}$  of normal noon sunlight.

*The Water Photometer.* Measurements of the light values for submerged plants requires a special type of the above photometer. This is water-tight, but the readings are made in the same manner as described above.

## ANGLE OF INCIDENCE OF LIGHT

The amount of light received by a leaf surface is of course influenced by the angle which the leaf makes with the axis of the incident rays. For leaves exposed to full sunlight, this is relatively unimportant because this angle changes each hour and day, with the altitude of the sun. The angle made during the early morning hours is balanced by that made in the late afternoon. For leaves which are shaded part of the day, the angles are more important. According to Clements<sup>2</sup> the extremes for light intensity are from 1.0 to 0.17 when the angle of incidence varies from 90° to 10°.

Of course only part of the light incident upon a leaf is available as a source of energy for photosynthesis, since the epidermis reflects and absorbs part of the light. By means of readings with sensitive paper, the amount of light received by the leaf, the amount which passes through the epidermis and the amount passing through the leaf may be found. From these data

<sup>2</sup> These readings were made where the axis of the incident radiation was normal to the epidermal surface.

the amount available for photosynthesis may be approximated. An epidermis coated with dense hairs or wax may screen out as much as 95 per cent. of the light falling on the leaf. Coloring matter in the epidermis serves as a light screen.

The following table shows the percentages of light absorbed by some of the leaves used in the later determinations.<sup>3</sup>

	TARAXACUM (DANDELION)	PHASEOLUS (BEAN)	HELIANTHUS (SUNFLOWER)
Percent. of total incident light passing through epidermis.	28.8	45.0	30.0
Percent. of total incident light passing through leaf.....	1.5	1.6	0.8
Percent. of total incident light absorbed in leaf.....	27.3	43.4	29.2

Since the epidermis of the dandelion reflects one-third more light than does that of the bean, the leaves of these plants are not doing photosynthesis with energy from the same amount of light, although they are growing in the same light exposure. Even for the same plant the light factor is very different in different parts. This is particularly true for the larger species, as trees. Hansen<sup>4</sup> found that the light intensity within the crown of ten common broad-leaved trees in August varied from 0.76 per cent. of full sunlight for *Acer saccharum* to 11.32 per cent. for *Quercus macrocarpa*. The light value in the greenhouse where *Malvaviscus arboreus* was growing was 50 per cent., while under the crown of this plant the light value was 2 per cent. Different parts of the *Taraxacum* rosette are exposed to very different intensities of light. The upper leaves of the plant measured received 50 per cent., those partly covered 10 per cent., while the very lowest ones received less than 1 per cent. of the light available.

#### QUALITY OF LIGHT

A Fuess hand spectroscope was used to determine the light quality, a standard spectrum being provided by a properly controlled electric lamp. By means of this arrangement it was possible to compare the intensity of any given portion of the spectrum obtained under a given glass or under a forest canopy, with that of the same portion of the standard spectrum.

#### PRELIMINARY OPERATIONS ON MATERIAL TO BE TESTED

##### METHODS OF KILLING PLANT MATERIAL FOR THE DETERMINATIONS

The main methods employed to prepare the plant material are the following:—

<sup>3</sup> Clements, F. E., *Plant physiology and ecology*. Henry Holt & Co.: New York. 1907. See page 60.

<sup>4</sup> Hansen, Herbert C. Leaf structure as related to environment. *Amer. Jour. Bot.* 4: 533-559. 1917. See page 538.

(1) Drying at 85° to 95°C. When ready for use the dried material is placed in water.

(2) Preserving the weighed material in 90-per-cent. alcohol. When ready for use the alcohol is evaporated off and water is substituted.

(3) Putting the weighed material into boiling water at once and then proceeding with the extraction.

(4) Keeping the leaf material in cold storage until ready for use and then extracting the sap with an hydraulic press. With methods 2 and 3, the relation of dry to fresh weight must be determined on separate samples so that the dry weight of the material used for a test may be calculated.

Since the chemical content of plant cells may be changed during the preserving process, through enzyme action, etc., the following comparative determinations were made, to find out what effect these processes might have on the final amount of photosynthate<sup>5</sup> indicated as present in the tissue. Leaves of *Bromus* and of wheat were used, being taken before the plants began to flower, so that they were young and growing rapidly.

	DRY WEIGHT OF LEAVES	TOTAL REDUCING SUGAR CALCULATED AS GLUCOSE	REDUCING SUGAR PER G. OF DRY WEIGHT
--	----------------------------	---	---

*Leaves of Bromus, 4 p.m., July 12*

	g.	mg.	mg.
Plants dried at 90°C.....	11.2416	128.0	11.38
Plants plunged into boiling water at once.....	11.3372	203.2	17.9
Plants frozen, sap expressed and boiled at once.....	14.1730	233.6	16.48
Plants frozen, sap expressed and then sap dried at 90°C.....	14.1730	197.8	13.95

*Minnesota Wheat no. 169, 5 p.m., July 16*

	g.	mg.	mg.
Plants dried at 90°C.....	6.665	105.6	15.8
Plants plunged into boiling water at once.....	5.20	120.8	23.2
Plants frozen, sap expressed and boiled at once.....	7.39	141.4	19.13
Plants frozen, sap expressed and then sap dried at 90°C.....	7.39	100.0	13.53
Plants not frozen nor dried, sap expressed and boiled at once.....	6.911	24.0	3.47
Plants not frozen nor dried, sap expressed and then sap dried at 80°C.....	6.911	21.3	3.08
Plants frozen for 20 hrs., then sap expressed and boiled at once.....	7.3915	19.2	2.50
Plants frozen for 20 hrs., then sap expressed and dried at 80°C.....	7.3915	18.4	2.48

<sup>5</sup> The term *photosynthate* is used in the present paper to denote the total reducing carbohydrate indicated by the method here used. It consequently refers mainly to water-soluble carbohydrates as shown by the digestion and extraction method described below. Where the word occurs it may be taken to mean total reducing sugar indicated, calculated as glucose. *EL.*

The greatest amount of photosynthate was shown where the fresh leaf material was put at once into boiling water. Leaves previously dried at 90°C. gave less photosynthate, which may have been due to enzyme action while heating up or to caramelization of the sugars. The material which was first frozen and then expressed gave a value lower than did that put at once into boiling water. The difference may be due to the fact that, even with very high pressure, some juice fails to be expressed; also, the chemical content of the expressed juice may not be exactly representative of the contents of the living cells.

#### MEASUREMENT OF LEAF AREAS

(1) Photographic blue-prints were made of the leaves to be tested, as soon as they were cut. From these the areas were calculated by (a) the planimeter method, or (b) by cutting out the leaf prints with scissors and weighing, the weight being compared with the weight of a measured area of the blue-print paper used.

(2) With a unit-area section-punch definite areas were cut out from the leaf and their combined weight was compared with that of the whole leaf.

#### DETERMINATION OF CARBOHYDRATES

##### GENERAL METHODS

Practically all the methods in use for the determination of carbohydrates are modifications of two general ones: (a) the optical method, with a polariscope, and (b) the chemical method, as with an alkaline solution of a copper salt. The optical method is not satisfactory for plant extracts because substances other than sugars in the solution may make the results inaccurate. The method here employed is a chemical one; it is described below.

##### THE METHOD HERE USED FOR THE DETERMINATION OF TOTAL REDUCING SUGAR<sup>6</sup>

*Extraction.* The dried leaf material is first ground and weighed. Two hundred cc. of distilled water is then added for each 5 g. or less of the ground material. If the material is to be used without drying it is cut into small pieces, in the fresh state. The material is next boiled for an hour, with a reflux condenser made by placing a funnel in the neck of an Erlenmeyer flask with a watch crystal on top. The boiling ruptures and gelatinizes starch grains and extracts sugars and starches. After cooling to room

<sup>6</sup> This method is a modification of the one described in: Official and provisional methods of analysis Association of Official Agricultural Chemists. U. S. Dept. Agric., Bur. Chem., Bull. 107 (revised), 1910. See p. 243.

temperature, 20 cc. of an aqueous solution of "Taka" diastase<sup>7</sup> is added, and the preparation incubated for 3-12 hrs. at a temperature of from 40°-50°C. A check for diastase action is run at the same time, with 5 g. of starch paste to which 20 cc. of the diastase solution has been added. When the viscosity of the paste becomes about like that of water, it may be assumed that sufficient time has elapsed for the conversion of all the soluble starch in the plant material. The material is boiled three times for an hour, each time with the subsequent addition of diastase and incubation for 3-12 hrs. After the third incubation, it is boiled to stop enzyme action, 5 cc. of a saturated aqueous solution of lead acetate is added, and the total volume of liquid is made up to 200 cc. The solid material of the leaves occupies space in the flask and correction must be made for it.<sup>8</sup> The acetate precipitates tannins, amino acids, etc., and thus clarifies the solution. To get rid of the excess of lead, 2-5 g. of anhydrous sodium carbonate is added. The precipitated lead carbonate is allowed to settle, a little more sodium carbonate is added and, if no precipitate forms as this passes through the supernatant liquid, the lead carbonate and fibrous material are filtered out.

To 150 cc. of the filtrate in an Erlenmeyer flask 15 cc. of hydrochloric acid (sp. gr., 1.125) is added. The solution is then heated 2.5 hours on a steam bath, to hydrolyze polysaccharides to monosaccharide sugars.

To the extract thus prepared, a drop of an alcoholic solution of phenolphthalein<sup>9a</sup> is added, and the solution is made almost neutral with aqueous sodium hydroxide solution. It is better to have the mixture slightly acid than to have it basic. After cooling it is made up to a volume of 200 cc.

*Carbohydrate Determination.* Fifty cc. of the extract is added to 50 cc. of Fehling's solution.<sup>10</sup> This is heated for six minutes in a beaker covered with a watch glass, in a bath of aqueous calcium chloride solution boiling at 108°C. Boiling should begin in four minutes and it should continue for two minutes. The mixture, with the copper oxide produced, is then poured into a specially prepared, weighed asbestos Gooch filter.<sup>11</sup> The precipitate

<sup>7</sup> One-tenth g. of "Taka" diastase (Parke, Davis and Co.) is "dissolved" in a liter of water. A few drops of toluene should be added to prevent bacterial infection; chloroform is not advised because it reduces the Fehling's solution later and introduces an error.

<sup>8</sup> Five g. soluble starch (Merck, according to Lintner) is mixed with 5 cc. of cold water. To this paste 15 cc. of boiling water is added and the whole mixture is then brought to boiling, forming a thick paste.

<sup>9</sup> "The average volume of fibrous material in 12 g. is 9 cc. The correction factor for 12 g. in 300 cc. is 0.97, and the percentage figures for reducing sugars are to be multiplied by this factor to obtain the true amounts." Leach, A. E. Food inspection and analysis. 3d ed. John Wiley & Sons: New York, 1913. P. 322.

<sup>9a</sup> Ten per cent. alcoholic solution of phenolphthalein, 10 g. in 100 cc. of 95 per cent. alcohol.

<sup>10</sup> Fehling's solution consists of equal volumes of the two following solutions, mixed just before using: (a) 31.63 g. crystalline copper sulphate dissolved in water to make 500 cc. of solution. (b) 173 g. sodium-potassium tartrate and 50 g. sodium hydroxide, dissolved in water to make 500 cc. of solution.

<sup>11</sup> The asbestos Gooch filter is prepared as follows: A perforated porcelain Witt plate is placed in the bottom of a Gooch crucible over which a disc of filter paper is laid. Over this a thin layer of fairly coarse asbestos suspended in water is poured. Another disc of filter paper is laid on, and then a layer of fine asbestos in water is added. Finally a suspension of purified barium sulphate in water is poured on to close the pores and stop leaks. The filter is washed thoroughly with water (to remove fine particles of asbestos in the bottom) with 10 per cent. ethyl alcohol, and then with 10 cc. of ether. Finally it is dried for thirty minutes at 100°C., cooled in

remaining on the filter is well washed with water at 70°C., followed by washing with 10 cc. of 90-per-cent. ethyl alcohol and then by washing with 10 cc. of ether. It is dried 30 minutes at 100°C., cooled in a desiccator and weighed. The tables of U.S. Dept. Agric. Bull. 107 (1910. Page 243) are used for calculating the dextrose equivalent of the copper oxide. The 50 cc. portion of the plant extract contains  $\frac{3}{16}$  of the total amount used, but correction must be made for the fibrous material, as already mentioned.

## BEHAVIOR OF REPRESENTATIVE HEALTHY PLANTS

### DAILY MARCH OF PHOTOSYNTHETIC ACTIVITY, AS INDICATED BY AMOUNTS OF REDUCING CARBOHYDRATE OBTAINED FROM THE TISSUES

*Phaseolus vulgaris*. Bean plants exposed in a greenhouse to full sunlight throughout the day, all of the same age, were employed to study the daily march of photosynthetic activity. Only the older compound leaves were taken. At the end of each hour, from 8 a.m. to 6 p.m., leaves were removed, photographic prints made and the leaves were killed in boiling water. Determinations of the photosynthate for each hour's material show the following results:—

TIME OF REMOVAL OF LEAVES	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR, AS GLUCOSE	REDUCING SUGAR PER SQ. CM. OF LEAF, ONE SIDE	HOURLY RATE OF INCREASE IN REDUCING SUGAR PER SQ. CM. OF LEAF DURING PRECEDING HOUR
	g.	sq. cm.	mg.	mg.	mg.
8 a.m.	1.6252	739.4	35.9	0.0485	—
9	2.8098	1510.6	80.0	0.0529	0.0044
10	1.1496	581.2	51.6	0.0887	0.0358
11	2.6700	1209.2	170.6	0.1410	0.0523
12 noon	11.5361	627.4	127.4	0.2030	0.0620
1 p.m.	1.7260	832.6	226.05	0.2715	0.0685
2	2.4504	1098.8	371.2	0.3378	0.0663
3	1.8430	780.3	314.7	0.4034	0.0656
4	2.2074	989.8	456.7	0.4614	0.0580
5	1.7016	746.3	370.6	0.4966	0.0352
6	1.4754	671.3	353.0	0.5250	0.0284

From these data it appears that the amount of reducing sugar increased continuously from hour to hour until 1 p.m. The hourly figures do not, of course, indicate the total amount of carbohydrate made during the period;

a desiccator and weighed. After use, the copper oxide is removed from the filter by dissolving in nitric acid (spec. grav. 1.42), the filter is washed with water, alcohol and ether, dried and used again.—To prepare the asbestos, digest amphibole asbestos with concentrated HCl (sp. grav. 1.18) for two days. Wash with water and digest for two days with 50 per cent. aqueous solution of sodium hydrate. Wash. Treat for three hours with hot Fehling's solution of the strength to be used in the sugar determinations. Wash the asbestos free from alkali, with water. Digest for three hours with nitric acid (sp. grav. 1.42) and then wash again with water until free from acid. Shake in water to separate the fibers, and keep under water. Since a large supply can be made at one time, the process of preparing asbestos does not have to be often repeated

some of the carbohydrates was utilized in respiratory and other activities within the leaf, and some was translocated to other parts of the plant body.

*Taraxacum taraxacum*. From dandelion plants exposed as in the case of the bean the following results were obtained, the leaves used being from the exposed part of the rosette:—

PERCENTAGE OF MIDDAY LIGHT INTENSITY	TIME OF REMOVAL OF LEAVES	AMOUNT OF FRESH LEAF MA- TERIAL USED	AREA OF LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR, AS GLUCOSE	REDUCING SUGAR PER SQ. CM. OF LEAF, ONE SIDE	HOURLY RATE OF INCREASE IN REDUCING SUGAR PER SQ. CM. OF LEAF DURING PRE- CEDING HOUR
		g.	sq. cm.	mg.	mg.	mg.
0.0	4 a.m.	1.2880	82.72	5.70	0.0689	—
3.5	6	1.1200	73.53	28.70	0.3890	0.3201
26.0	8	1.6334	98.00	91.40	0.9326	0.5436
64.0	10	1.7746	97.60	153.00	1.5678	0.6352
100.0	12 noon	1.9494	52.47	117.62	2.2416	0.6738
90.0	2 p.m.	1.9970	80.80	239.00	2.9580	0.7164
32.0	4	1.7004	91.51	154.20	1.6850	-1.2730
4.5	6	1.5234	76.17	44.50	0.5842	-1.1008

At 4 a.m. the leaves were practically depleted showing only 0.0689 mg. of reducing sugar per sq. cm. The quantity of photosynthate increased rapidly until 2 p.m. when a maximum of 2.9580 mg. of sugar per sq. cm. was found. After this time the total photosynthate present decreased each hour. The decrease seems to indicate that rapid translocation must have been taking place. The very large amount of sugar present at 2 o'clock, compared with the maximum found in other plants in these experiments, may be accounted for by the fact that these plants were out-doors, exposed to full sunlight instead of to 50 per cent. of full sunlight, which was received by the plants in the greenhouse.

#### RELATION OF LEAF POSITION TO PHOTOSYNTHETIC ACTIVITY

The following experiment was planned to compare photosynthetic activity in leaves in the usual position and in inverted leaves of the sunflower.

In the leaves of this plant (*Helianthus annuus*), the palisade tissue, containing the chloroplasts, is located just under the upper epidermis. Next to the lower epidermis is the layer of sponge tissue in which there are few chloroplasts.

With leaves in the usual position most of the light reaching the palisade cells passes merely through the epidermis, while with inverted leaves most of the light comes to the palisade only after penetrating through the sponge tissue. Some of the leaves of a sunflower plant in the greenhouse were left in the usual position and some were inverted, by twisting the petioles, and were supported in that position with pieces of Bristol board. In this plant the leaves are so well separated from each other on the stem that there is

practically no shading of one leaf by an adjoining one. At 5 p.m. the leaves of each group were gathered and dried separately. The following carbohydrate determinations were subsequently secured:—

		AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAF, ONE SIDE	REDUCING SUGAR, AS GLUCOSE	
				Total	Persq. cm. of leaf, one side
		g.	sq. cm.	mg.	mg.
Sunflower leaves in usual position	Lowest leaf.....	0.4132	143.3	21.3	0.148
	Third leaf.....	0.8140	239.5	25.6	0.107
	Fourth leaf.....	0.6548	204.3	17.8	0.087
	Sixth leaf.....	0.8526	207.5	21.3	0.103
	Eighth leaf.....	0.9269	222.6	41.1	0.185
	Highest leaf.....	1.1822	278.9	117.1	0.0421
Sunflower leaves in inverted position	Second leaf.....	0.2060	65.4	14.8	0.226
	Fifth leaf.....	0.5166	177.6	30.0	0.167
	Seventh leaf.....	0.8096	214.9	21.7	0.100

These results indicate that the small leaves at the top of the plant had a relatively low carbohydrate content. This may not indicate low photosynthetic activity, but may be due to the fact that these leaves are growing most rapidly and use the carbohydrates as these are made. From the data given, no correlation is apparent between position of the leaves on the stem and the amount of reducing sugar shown. The wide variation in the results may have been partly caused by the shade cast by the framework of the greenhouse at different times of the day on different leaves. The top of the plant reached almost to the greenhouse roof and the proximity to the glass may also have affected the results for the upper leaves.

The inverted leaves generally showed a higher value for reducing sugar than did those in the usual position. An explanation may be that twisting the petiole in inverting the leaves retarded the outward movement of sugar and more than balanced the lowered photosynthetic rate brought about by the decrease in light intensity caused by the screen of sponge tissue.

#### COMPARATIVE PHOTOSYNTHETIC ACTIVITY OF LEAVES OF DIFFERENT LAYERS IN A ROSETTE PLANT

The leaves of a rosette plant which are in the top layer are usually exposed to full sunlight, while those of the lower layers are exposed to greatly reduced light intensities. Most of the leaves in the lower layers have localized areas here and there that are exposed to full light and other areas that are somewhat shaded, while those portions next to the stem receive practically no light at all. At 5 p.m. leaves of dandelion were taken and a comparison was made between those of three different layers in the rosette, with regard to

the amounts of reducing sugars indicated after about eight hours of sunshine. The results follow:—

	PERCENT- AGE OF FULL LIGHT INTENSITY	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	REDUCING SUGAR, AS GLUCOSE	
				Total	Persq.cm. of leaf, one side
		g.	sq. cm.	mg.	mg.
Leaves in upper layer of rosette .....	50	3.2878	972.0	104.4	0.1198
Lower leaves of rosette.....	10	2.601	1255.0	61.8	0.0494
Leaves next to the soil.....	1	1.038	496.0	24.1	0.0458

The very small values shown for the leaves next to the soil may be related to the fact that these leaves are generally the first to become etiolated. When the rosette is young these lowest leaves are in a position to receive strong light and they develop under these conditions. As the upper layers of leaves are progressively added light is more and more cut off from the lower layers.

#### RELATION OF LIGHT INTENSITY TO PHOTOSYNTHETIC ACTIVITY

To obtain an idea of the relation between light intensity and the amount of reducing sugar shown by leaves after prolonged exposure, some preliminary tests were made with *Phaseolus vulgaris*. Three different degrees of light intensity were employed, for different plants. The plants of one group were exposed to the full light of the greenhouse, whose intensity proved to be 50 per cent. of that of full sunlight, and those of the two other groups were shaded by white cotton cloth so as to receive 10 and 0.3 per cent. of the full sunlight as this was measured by the Clements photometer. Determinations of the reducing sugars obtained from the leaves of these plants were made for 4 o'clock on May 15, 1917. The results follow:—

PERCENTAGE OF FULL LIGHT INTENSITY	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	REDUCING SUGAR, AS GLUCOSE		
			Total	Per sq. cm. of leaf, one side	
				Actual	Relative
	g.	sq. cm.	mg.	mg.	
50.0	1.0086	620.24	265.4	0.4278	100
10.0	0.6688	401.70	11.5	0.0261	6
0.3	0.7290	437.70	0.4	0.0091	2

In the medium light, where the intensity was  $\frac{1}{5}$  of that of the strongest light, only  $\frac{1}{16}$  as much reducing sugar was found; but the weakest light, whose intensity was  $\frac{1}{100}$  of that of the strongest light, gave  $\frac{1}{50}$  as much reducing sugar as was shown for the strongest light. For the amount of light re-

ceived, relatively more reducing sugar was found in the leaves exposed to the weakest light than was found in those exposed to the medium light.

#### PHOTOSYNTHETIC ACTIVITY AS RELATED TO LIGHT QUALITY

The following experiment was devised to study the effects of differently colored lights on photosynthesis. Wooden frames or chambers, painted white inside and out, with colored glass tops, were used like ordinary cold-frames. These stood in the greenhouse and the glass top sloped at an angle of about 45° toward the south. The panes of glass, all of similar thickness, were fitted into grooves on the upper margins of the chamber walls, so that they could be slipped in and out. Red, yellow, blue and green glasses were used. The transmission spectrum of each glass was examined with a spectrophotometer and found to be practically what the apparent color of the glass indicated. The red glass screened out the blue and some of the green. The yellow glass cut out the blue and a little of the red. The green glass absorbed part of the blue and most of the red, indicating that its true color was not as much green as blue. No suitable colorless glass was available, so the control plant was grown in the greenhouse next to the colored light chambers, but without any chamber. All of the light reaching any of the chambers had first passed through the glass of the greenhouse roof.

Phaseolus seedlings were placed in the chambers when two weeks old and all were kept well watered during the experiment. The plants in all cases grew rather tall and slender, probably due to the lowered light intensity caused by the greenhouse roof and the glass of the chamber. The days of the experiment were very warm and the sky was clear. Until the stored material in the cotyledons was exhausted little difference was noted between the seedlings of the different chambers. The development of the first compound leaves was more rapid, the simple leaves were larger and the general vigor of the plant appeared to be greater, for those grown under the yellow and red glasses than for those grown under the blue and green glasses. When the plants were four weeks old leaf samples representing each light treatment were taken, at 2 p.m., and determinations of the reducing sugar were made in the usual way. The results are given below:—

GLASS SCREEN	AMOUNT OF FRESH LEAF MATERIAL USED	AREA LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR, AS GLUCOSE	REDUCING SUGAR, AS GLUCOSE, PER SQ. CM. OF LEAF, ONE SIDE
	g.	sq. cm.	mg.	mg
Greenhouse roof alone.....	1.682	541	96.3	0.1780
Greenhouse roof and red glass .....	1.207	494	71.8	0.1454
Greenhouse roof and yellow glass.....	1.382	521	70.2	0.1340
Greenhouse roof and green glass .....	2.192	793	68.4	0.0862
Greenhouse roof and blue glass.....	0.935	427	34.4	0.0805

From these values it appears that of the four chambers, that with red glass was the most efficient in producing reducing sugar in the leaves. The smallest amount of reducing sugar per unit of leaf area was obtained from the chamber with blue glass. Leaves from the chamber with green glass showed but little more sugar.

RELATION OF SUBMERGENCE UNDER WATER TO PHOTOSYNTHETIC ACTIVITY

Some tests were made with *Philotria canadensis*, to determine the relation of depth of submergence under water to the amount of photosynthate found in the leaves. The stems were submerged to a depth of from 25 to 30 cm. and extended to the surface of the water. The leaves on the most deeply submerged parts were usually etiolated, while the upper leaves were of a good green color. With the water photometer light readings were taken at several depths in the jars in which these plants were growing, these being in the greenhouse. Leaves taken from these depths showed the following results:—

DEPTH OF SUBMERGENCE	PERCENT- AGE OF TOTAL LIGHT INTENSITY	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	REDUCING SUGAR, AS GLUCOSE		
				Total	Per sq. cm. of leaf, one side	
					Actual	Relative
		<i>g.</i>	<i>sq. cm.</i>	<i>mg.</i>	<i>mg.</i>	
Surface of water . . . . .	40	0.3068	543	69.5	0.128	100
12.5 cm. below surface.....	30	0.3770	426	47.7	0.112	87
20 cm. below surface. ....	20	0.3582	334	33.6	0.100	78

According to these data, the amount of reducing sugar found was less with greater depths of submergence, being nearly inversely proportional to the depth and almost directly so to the light intensity actually received. The first few centimeters below the water surface receive comparatively large percentages of the original light. It is probable that there is a very rapid decrease in photosynthetic activity with increasing depths up to perhaps 30 cm.

TRANSPIRING POWER AS RELATED TO PHOTOSYNTHETIC ACTIVITY

To gain some idea of the relation that may hold between the transpiring power of the plant and its photosynthetic activity, an experiment bearing on this relation was carried out with the three species, *Equisetum fluviatile*, *Helianthus annuus* and *Phaseolus vulgaris*.<sup>12</sup>

<sup>12</sup> This work was done in cooperation with Miss Louise Dossdall, who obtained the transpiration data.

The plants were grown in the greenhouse, in soil kept saturated with water. The morning of the experiments the pots were placed in Ganong aluminium shells<sup>13</sup> filled with water. Evaporation from the soil was avoided by covering it with a layer of waxed paper, held in place by the movable ring at the top of the shell. The space between the plant stems and the paper was closed by sealing with a mixture of paraffine and vaseline, such as that described by Briggs and Shantz.<sup>14</sup>

The plants all stood side by side, so that they were all exposed to about the same environmental conditions as far as transpiration and photosynthesis were concerned. For all three species the amount of water lost during the period from 7:30 a.m. to 5:30 p.m. was determined by weighing and these amounts are taken as relative measures of the transpiring powers of the different plants as such. Corresponding determinations of the increase in reducing sugar were also made, the test material being stems in the case of *Equisetum* and leaves in the case of the other two plants. These determinations involved tests with material collected at 7:30 a.m. and with other material collected at 6:30 p.m. For *Helianthus* and *Phaseolus*, additional material was collected at noon, and subjected to the determination of its reducing sugar. The sugar determinations for 5:30 p.m. were made with material from the same individual plants as were used for the transpiration measurements; other, but similar, plants furnished the material for the morning and noon sugar determinations. Leaf prints were made for areal determinations for *Helianthus* and *Phaseolus*, but the area of the *Equisetum* stems were calculated from measurements. Finally, the transpiring powers and the photosynthetic rates (photosynthetic powers) were all expressed in terms of a unit area of the exposed surface. Records of air temperature and air humidity were obtained at the times of observation. These records are given below.

TIME OF OBSERVATION	RELATIVE AIR HUMIDITY	AIR TEMPERATURE	
	<i>per cent</i>	<i>Deg. C.</i>	<i>Deg. F.</i>
7.30 a.m.	70	21.1	70
12 noon	28	25.5	78
5.30 p.m.	20	29.5	85

The transpiration data are given below, being for the period from 7:30 a.m. to 5:30 p.m., May 4, 1917:—

<sup>13</sup> Ganong, W., New normal appliances for use in plant physiology. Bot. Gaz. 41: 209-213. 1906. See p. 212.

<sup>14</sup> Briggs, L. J., and H. L. Shantz. A wax seal method for determining the lower limit of available soil moisture. Bot. Gaz. 51: 210-219. 1911.

PLANT	PLANT NO.	AREA <sup>a</sup> OF EXPOSED SURFACE	TOTAL WATER LOSS	TOTAL WATER LOSS PER SQ. CM. OF EXPOSED SURFACE	WATER LOSS PER SQ. CM. OF EXPOSED SURFACE	
					Actual	Relative
		<i>sq. cm.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>	
<i>Equisetum</i> .....	1	38	5.3	0.139	} 0.152	1.00
	2	68	10.0	0.147		
	3	30	5.1	0.170		
<i>Helianthus</i> .....	1	220	21.3	0.097	} 0.088	0.64
	2	315	20.0	0.092		
	3	391	40.8	0.104		
<i>Phaseolus</i> .....	1	651	48.7	0.075	} 0.065	0.43
	2	882	53.5	0.061		
	3	347	20.0	0.058		

<sup>a</sup> Because of the different light relations of the exposed surface of *Equisetum* and that of *Helianthus* and of *Phaseolus*, total surface was measured from *Equisetum* and only the upper leaf surface for *Helianthus* and for *Phaseolus*.

The results of the determinations of reducing sugars are shown in the following tabulation:—

PLANT	TIME OF COLLECTING MATERIAL	AMOUNT OF DRIED LEAF MATERIAL USED	EXTENT OF EXPOSED SURFACE	TOTAL REDUCING SUGAR, AS GLUCOSE	REDUCING SUGAR, AS GLUCOSE, PER SQ. CM. OF EXPOSED SURFACE	GAIN IN REDUCING SUGAR PER SQ. CM.	
						Actual	Relative
		<i>g.</i>	<i>sq. cm.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	
<i>Equisetum</i> .....	7:30 a.m.	0.2100	60.0	17.4	0.290	—	1.0
	5:30 p.m.	0.2884	68.0	30.9	0.440	0.150	
<i>Helianthus</i> .....	7:30 a.m.	1.0446	378.5	2.0	0.0052	—	1.9
	12 noon	0.9354	290.7	33.0	0.1135	0.1083	
	5:30 p.m.	0.6674	268.0	76.5	0.2854	0.2802	
<i>Phaseolus</i> .....	7:30 a.m.	1.5090	754.0	126.7	0.1681	—	3.0
	12 noon	1.0150	518.0	272.4	0.5259	0.3578	
	5:30 p.m.	2.4540	767.0	472.4	0.6172	0.4491	

For the full 10-hour period, from 7:30 a.m. to 5:30 p.m., the *Helianthus* plant showed 1.9 times, and the *Phaseolus* plant 3.0 times, as much photo-synthetic activity as did the *Equisetum* plant, per unit of the exposed surface considered. The relatively low activity of *Equisetum* may be partially or wholly related to the direction of exposure of the light-absorbing and carbon-dioxide-absorbing surfaces in this plant. Here the stem surfaces are the ones involved, and these are cylindrical and vertical, while the ab-

sorbing surfaces of the other two forms (leaf surfaces) are to be regarded as plane and nearly horizontal. It may thus be that the *Equisetum* stems differ from the leaves of the other plants by absorbing radiation mainly in the morning and late afternoon, when the direct sunlight approaches being horizontally incident, and when it is already markedly weakened by the absorption occurring in the deep atmospheric layer which it has traversed.

*Helianthus* epidermis screens out 70 per cent. of the light incident upon it, while *Phaseolus* screens out 45 per cent. This means that *Helianthus* actually receives 30 per cent. of the light, and *Phaseolus* 55 per cent. *Phaseolus* thus receives 1.9 times as much light as *Helianthus* per unit of exposed surface, and produces 1.6 times as much photosynthate, so that while the former is apparently the more active photosynthetically, it has a somewhat lower efficiency when the light really received is considered.

Turning to the relation between transpiring power and photosynthetic power, the two tabulations given just above show that the three plants (*Equisetum*, *Helianthus*, *Phaseolus*) have transpiring powers in the proportions 1.00: 0.64: 0.43, while they have photosynthetic powers in the proportions 1.00: 1.9: 3.0, relative to a unit of exposed surface here considered, in all cases. If both leaf surfaces are considered for the last two plants, to compare with the total stem surface used for *Equisetum*, then these proportions become:—1.00: 0.95: 1.50.

#### PHOTOSYNTHETIC ACTIVITY OF STAMENS

Some tests were made regarding photosynthesis in the stamens of *Acer negundo*. It is frequently supposed that the presence of chlorophyll indicates capacity for photosynthesis, under suitable conditions, no matter in what part of the plant it may be located, and this idea was in mind when these tests were planned. The flowers were separated from the bud scales and then killed and tested, a test being made for the morning and for the evening condition, on each of two successive days. The results follow:—

TIME OF REMOVAL OF STAMENS		DRY WEIGHT OF STAMENS USED	TOTAL REDUCING SUGARS, AS GLUCOSE	REDUCING SUGAR PER G. OF DRY WEIGHT	GAIN IN REDUCING SUGAR PER G. OF DRY WEIGHT PER DAY
		g.	mg.	mg.	mg.
May 5	8 a.m. ....	1.8310	119.7	65.4	—
	6 p.m. ....	1.3520	98.1	74.1	8.7
May 6	8 a.m. ....	0.9276	57.56	62.1	—
	6 p.m. ....	1.1723	81.55	69.7	7.6

These results show that, for both of the days considered, there was a slight gain in reducing sugar as shown by this method, the amount shown being somewhat larger for the evening tests than for the morning ones. This may indicate that such stamen filaments as these may produce a small amount of carbohydrate during the period of flowering.

## RELATION OF PARASITES TO PHOTOSYNTHETIC ACTIVITY

Some tests were made to find out whether the yield of reducing sugar, as shown by the method here employed, might be related to the presence or absence of parasites. These tests dealt (1) with artificial infections of *Avena sativa* with *Puccinia coronata* and of *Triticum hordeum* with *Puccinia graminis*; (2) with natural infections of *Arisaema triphyllum* with *Uromyces caladii*, of *Aquilegia vulgaris* with *Erysiphe cichoriacearum*, and of *Swainsonia* sp. with *Erysiphe*; (3) with *Phaseolus vulgaris* infected with *Tetranychus telarius* and *Phaseolus vulgaris* infected with *Aphis* sp. These experiments are considered below.

### ARTIFICIALLY INFECTED AVENA AND TRITICUM

*Preparation of Material for the Tests.* "Blue Stem" wheat (Minnesota No. 169) and "Improved Ligowa Oats" (Minnesota No. 281) were the varieties used in the rust experiments here described. The seeds were planted in pots of a somewhat sandy loam containing a large admixture of leaf-mold. In from 7 to 10 days, in winter, the seedlings were from 5 to 7 cm. high and the first one or two leaves were unrolled. The cultures were then thinned so as to leave ten plants in each pot, and all but one of the leaves (the outer one) were removed from each plant. A film of water was formed over the leaf by means of a fine spray. A sterilized dissecting needle flattened at the side, was used to transfer the spores to the leaves of the seedlings to be infected. The needle was first wetted and rubbed over a pustule to collect spores. When loaded with spores it was then gently rubbed over the lower surface of the leaf to be infected. After the transfer, the pot of inoculated seedlings was set in a pan of water, and covered with a bell jar. The seedlings were kept 48 hours in the saturated atmosphere thus secured, to insure spore germination. Paper was spread above the jars to prevent injury from too intense radiation. The plants were then taken from the bell jars and kept at a temperature of about 70°C., with an air humidity of about 65 per cent., in the greenhouse. The first evidence that infection had taken place was the appearance of pale spots on the leaf surface, and in from seven to nine days, in the winter months, the rust pustules became well defined. During the experiments carried on in May, the seedlings were ready for inoculation in six days, and infection became apparent after five more days. During the next two or three days the pus-

tules appeared as small brown spots, which enlarged and finally became crumpled, discharging spores. It was found convenient to plant seeds each time seedlings were inoculated, so that a supply of seedlings was at the proper age for inoculation when the spores from the last series to be inoculated were mature.

During the winter, when the experiment was first undertaken, great difficulty was experienced in getting infection. In the first experiment attempted, the pot, after inoculation, was set on a bench in a layer of wet cinders. The plant and pot were surrounded by panes of glass and a pane of glass was laid over the top of the enclosure thus formed. This enclosure kept the air surrounding the plants comparatively moist, with a relative humidity of from 85 to 90 per cent., but the air never became very nearly saturated because air currents passed between the panes. Only 2 per cent. of infection resulted under these conditions. Bell jars were then substituted for the panes of glass, the pots were set in pans of water instead of cinders, and they were left in pans of water after the bell jars were removed. 40 per cent. of infection resulted under these conditions, but even this was not regarded as satisfactory. A higher percentage of infection took place with *Puccinia coronata* under these conditions than with *Puccinia graminis*. The greenhouse in which these plants were grown had a relative air humidity ranging from about 70 per cent. at midnight to about 30 per cent. at 3 p.m. The next series of plants were placed in the aquatic greenhouse when removed from the bell jars. Here the air humidity was constantly high, averaging about 70 per cent. 100 per cent. of infection resulted in this case, when the days during which the rust developed were sunny.

When the pots were removed to the dark room after the incubation period was passed, only a small percentage of infection resulted, the pustules were not as large and the appearance of the infection was delayed by the length of time the plants were in the dark. In May the greenhouse glass was coated with white lead on a day of intense sunshine and high temperature, and the weeks following this were cool and more or less cloudy. The coating on the glass decreased the light value within the greenhouse from 50 per cent. to 13 per cent. of the full sunlight value. The seedlings grew very tall and slender under this set of conditions. Plants which were grown with the 50-per-cent. light and inoculated the day the light intensity was reduced, showed a marked difference from the others in the appearance of the sori. Instead of large pustules (2-5 mm. in length for *P. graminis* and 1-2 mm. long for *P. coronata*, these were very small, 0.1 mm. or less in diameter. In these cases the infection was only 70 per cent. of that obtained under the greater light intensity. The green areas at the centers of the etiolated regions of infection were more distinct with the weaker light.

*Determinations of reducing sugar in rusted plants.* When the infected plants were in suitable condition for the tests, four kinds of material were

gathered for the determinations of reducing sugar: (1) seriously infected leaves in which the pustules were not yet crumpeut; (2) similar leaves with crumpeut sori; (3) the excised infected areas from seriously infected leaves; and (4) the uninfected regions of seriously infected leaves. Besides these four kinds of material, representative healthy leaves from uninfected plants grown under the same general conditions as the infected ones, but not inoculated, were also included. By infected regions is meant the pustules and the surrounding etiolated regions, as shown by ordinary appearance. For the special study of infected tissues these regions were cut out with scissors and killed, their area was determined by the print method, and the material was then dried. The remaining parts of the same leaves were used for the uninfected regions. The infected region always included the tip of the leaf and its upper portion, while the uninfected region included the parts next to the stem, the younger, growing portions.

The results obtained from the determinations of reducing sugar in these tissues are shown in the tabulation below.

HOST AND PARASITE	MATERIAL USED	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR AS GLUCOSE	REDUCING SUGAR, AS GLUCOSE, PER SQ. CM. OF LEAF, ONE SIDE
		g.	sq. cm.	mg.	mg.
Avena infected with <i>Puccinia coronata</i>	Uninfected leaves.....	0.2014	122.23	30.7	0.2511
	Leaves seriously infected, sori not crumpeut.....	0.4660	276.50	50.3	0.1820
	Leaves seriously infected, sori crumpeut.....	0.5380	309.02	36.7	0.1187
	Infected regions of infected leaves.....	0.2710	157.70	3.5	0.0221
	Uninfected regions of infected leaves.....	0.1928	113.56	11.5	0.1011
	Uninfected leaves.....	0.2620	103.04	32.5	0.3876
Triticum infected with <i>Puccinia graminis</i>	Leaves seriously infected, sori not crumpeut.....	0.1700	59.09	10.5	0.1942
	Leaves seriously infected, sori crumpeut.....	0.1814	65.02	10.2	0.1538
	Infected regions of infected leaves.....	0.2263	79.32	1.0	0.0120
	Uninfected regions of infected leaves.....	0.1839	67.28	9.9	0.1472
	Uninfected leaves.....				

These data indicate that the fungus interfered seriously with the accumulation, in the tissues, of materials that appear as reducing sugars. A comparison of crumpeut regions with regions infected, but with non-crumpent sori, suggests that the breaking out of the spores reduces the ability of the

host tissues to make carbohydrates more than 50 per cent. This may possibly be due to interference with transpiration caused by the rupturing of the leaf epidermis accompanying spore formation. Also, as the spores mature the parasite may withdraw more nutrient material from the surrounding regions of the leaf, thus depleting the supply of stored food in the latter.

The central, green portion of an infected region in such leaves as these, in the earlier stages of infection, evidently contains chlorophyll. It may be that the processes that destroy the chlorophyll in the etiolated region about this mass of green tissue (which contains much of the fungus mycelium) may be prevented in this central region, where the spores are subsequently formed. Just how much photosynthetic activity may occur in the central green regions cannot be conjectured; the green regions are so small that their separation from the surrounding etiolated regions was not attempted.

#### ACCIDENTALLY INFECTED ARISAEMA, AQUILEGIA AND SWANSONIA

*Arisaema triphyllum* infected with *Uromyces caladii*. The *Arisaema* material was derived from greenhouse-grown plants that were found to be infected with this rust. The leaves were cut and killed at the end of a sunny day, and the usual tests were made. The results follow:—

MATERIAL USED	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR, AS GLUCOSE	REDUCING SUGAR, AS GLUCOSE, PER SQ. CM. OF LEAF, ONE SIDE	
				Actual	Relative
	g.	sq. cm.	mg.	mg.	
Uninfected leaves.....	2.4030	929.5	104.1	0.112	100
Infected leaves, pustules covering surface but still white, not crumpled.....	2.7608	920.3	79.97	0.0869	88
Infected leaves, pustules covering surface and spores yellow.....	2.3854	795.1	35.38	0.0445	40

From these data it appears that the earlier stages of pustule formation were accompanied by reduced photosynthetic activity but this reduction was more marked with the fungus in the crumpled stage, where the rate of leaf transpiration was probably affected.

*Aquilegia vulgaris* infected with *Erysiphe* and *Swainsonia* sp. infected with *Erysiphe* sp. The leaf material used was collected by means of a leaf-punch, in the greenhouse at the end of the day. The infected leaves were not etiolated. The data are shown below:—

HOST AND PARASITE	MATERIAL USED	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR, AS GLUCOSE	REDUCING SUGAR, AS GLUCOSE PER SQ. CM. OF LEAF ONE SIDE	
					Actual	Relative
		g.	sq. cm.	mg.	mg.	
Aquilegia in- fected with Erysiphe	Uninfected leaves . . . .	1.4724	598.5	312.8	0.5226	100
	Infected leaves, my- celial areas cover- ing about half of leaf . . . . .	1.3086	531.9	263.4	0.4952	95
	Infected leaves, my- celium covering en- tire leaf . . . . .	1.3640	554.4	247.8	0.4469	86
Swainsonia in- fected with Erysiphe	Uninfected leaves . . . .	1.056	283.5	168.8	0.5096	100
	Infected leaves my- celium covering leaf	1.512	409.8	114.6	0.2790	55

The results shown above appear to indicate that the parasites interfered seriously with the process of photosynthesis in these cases. The effect of *Erysiphe* on leaves of *Aquilegia* was much less marked than on those of *Swainsonia*.

When the *Uromyces* pustules on *Arisaema* leaves were white the leaf epidermis was unbroken, but the cells of the host were broken and crowded by the fungus in the region of the infection. This may account, at least in part, for some decrease in the amount of reducing sugar shown for the early stages of infection. When the spores became yellow the epidermis was broken, and transpiration must have been greatly increased. The drying out of the leaf that may have resulted from the rupture of the epidermis, may partly or wholly account for the very marked decrease in reducing sugars for this later stage.

In the plants infected with *Erysiphe*, the superficial mycelium must have decreased the light intensity for the infected regions and this, together with the activity of the parasite in withdrawing food from the tissues of the host, may account for the apparent lowering of carbohydrate content of the infected leaves.

#### PHASEOLUS VULGARIS INFESTED WITH ANIMAL PARASITES

Plants of *Phaseolus vulgaris* growing in the greenhouse and found to be infested with *Tetranychus telarius* (red spider) and *Aphis* sp., were employed to obtain some idea of how the presence of such animal parasites may influence the amount of reducing sugar found in leaves at the end of a bright day. In the case of *Phaseolus vulgaris* infested with red spider, two classes

of leaves were tested, those that were seriously infested and others upon which the parasites were much less numerous. *Phaseolus* leaves seriously infested with *Aphids* were used. In both cases uninfested leaves, from uninfested plants, were employed as controls. The results are tabulated below.

PARASITE	MATERIAL USED	AMOUNT OF FRESH LEAF MATERIAL USED	AREA OF LEAVES USED, ONE SIDE	TOTAL REDUCING SUGAR AS GLUCOSE	REDUCING SUGAR, AS GLUCOSE PER SQ. CM. OF LEAF, ONE SIDE
		g.	sq. cm.	mg.	mg.
Tetranychus telarius	Uninfested leaves	1.6820	541	261.3	0.483
	Somewhat infested leaves.....	1.8430	749	228.4	0.305
	Badly infested leaves	2.1040	821	155.8	0.189
Aphids	Uninfested leaves.....	1.2594	593	264.70	0.462
	Badly infested leaves	0.7180	322	110.76	0.344

It appears from the values just given that marked differences may be detected between the reducing sugar contents of uninfested leaves of *Phaseolus vulgaris* and that for leaves more or less seriously infested with "red spider." In the two tests here considered, the more serious was the infestation, the more the yield of reducing sugar from the leaves was decreased, this decrease being 37 per cent. in the case of the less serious infestation and 61 per cent. in the more serious one. Of course the most obvious suggestion from these results is that the insects withdraw carbohydrates (and any other substances that might result in reducing sugars by the method here used) from the leaves. It is also possible, of course, that some portion of the observed effect of infestation may be due to shading of the leaves by the bodies of the insects and also by the web of silk that these animals produce. It may be suggested, furthermore, that the silk web, as well as the insects themselves may alter the transpiration rate.

The leaves infested with aphids showed numerous, scattered, small white spots marking the regions where the sucking organs of the insects had been in operation. It appears that a small fraction of the whole chlorophyll-bearing tissue of the leaf is thus robbed of its chlorophyll. This may reduce the photosynthetic power of the leaf to some extent. Also, of course, the presence of the insects on the leaf surface must alter the intensity and quality of the light received by the latter and must exert some influence on the rate of transpiration. The insects must be supposed to remove some carbohydrates, etc., from the leaf tissues, which alone may perhaps account for the slightly smaller amount of reducing sugar obtained from the infested leaves, as compared with that derived from the uninfested ones.

## GENERAL CONCLUSION

The primary object of this investigation was to devise a more rapid and satisfactory method for determining the comparative photosynthetic activity of different plants and plant parts and of the same plants, etc., under different sets of conditions or at different times. This is thought to have been accomplished. A number of other methods were tried but none proved apparently satisfactory except the one here described. The results of its application in a wide range of representative cases are sufficiently numerous and consistent with themselves and with general probability to warrant the conclusion that this method has considerable value. A summary statement of the work is presented in the Abstract, at the beginning of this paper.

## LITERATURE CITED.

- BRIDGES, L. J. AND H. L. SHANLEY. A wax seal method for determining the lower limit of available soil moisture. *Bot. Gaz.* 51: 210-219. 1911.
- CLEMENTS, F. E. *Plant physiology and ecology.* Henry Holt & Co.: New York, 1907.
- GANNONG, W. New normal appliances for use in plant physiology. *Bot. Gaz.* 41: 209-213. 1906.
- HANSON, HERBERT C. Leaf structure as related to environment. *Amer. Jour. Bot.* 4: 533-559. 1917.
- LEACH, ALBERT E. *Food inspection and analysis.* 3rd ed. John Wiley & Sons: New York, 1913.
- Association of Official Agricultural Chemists. *Official and provisional methods of analysis.* U. S. Dept. Agric. Bur. Chem. Bull. 107 (revised). 1910.

# A COMPARATIVE STUDY OF THE TWO RACES OF RHIZOPUS NIGRICANS<sup>1</sup>

GRACE A. DUNN

## ABSTRACT<sup>2</sup>

This paper presents the results of an experimental study of the physiological characteristics of Blakeslee's two races, male and female, of *Rhizopus nigricans*. In most of the experiments liquid culture media were employed with 100-cc., flat-bottomed "Pyrex" glass flasks containing 50 cc. of solution. Temperature was maintained practically constant and light was excluded; only the osmotic and chemical properties of the medium differed from culture to culture. The rate of growth was measured in terms of the dry weight produced during the culture period. The results obtained have a direct bearing upon the general problem of the nutritional requirements of fungi and they give indications as to the nature of the medium required for optimal vegetative growth and spore production in these two races of *Rhizopus*. The main conclusions are stated in the following paragraphs.

(1) Both races thrive excellently in a liquid medium containing the salts  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4$ ,  $\text{FePO}_4$  and dextrose, if the proper partial concentrations are employed.

(2) The element calcium is apparently not needed if the solutes just mentioned are used. On the other hand,  $\text{Ca}(\text{NO}_3)_2$  is not noticeably injurious to these organisms, in the partial concentrations tested, at least with a fair physiological balance of the other solutes.

(3) With the salt and dextrose combinations tested, the  $\text{NO}_3$ -ion appears to be without any notable influence, and the  $\text{NO}_2$ -ion (tested as  $\text{KNO}_2$ ) appears to belong in the same category. For the conditions studied it appears that these races are unable to derive their nitrogen supply from either  $\text{NO}_3$  or  $\text{NO}_2$ , but they do utilize the ion  $\text{NH}_4$  when supplied as  $\text{NH}_4\text{NO}_3$ .

(4) No study was carried out regarding the particular necessity for the other ions in the solutions employed, but it seems probable that the necessary ions for good vegetative growth and sporangia production are K,  $\text{NH}_4$ , Mg, Fe,  $\text{PO}_4$  and  $\text{SO}_4$ .

(5) Glycerine is the only carbon compound besides dextrose that received attention, and, although it supports fair growth when accompanied by the other solution conditions mentioned above as favoring good growth with dextrose, still it is not nearly so favorable to these organisms as is dextrose.

(6) For both races the physiological influence of salt proportions, in the solutions tested, is different with different combinations of total-salt concentration and dextrose concentrations. Likewise, as should be expected, the influence of total-salt concentration is definitely controlled by the particular salts used, their relative partial concentrations, and the dextrose concentration. Finally, the influence of the partial concentration of dextrose in these solutions is a function of salt proportions and total-salt concentration. In short, for the other environmental conditions employed in this

<sup>1</sup> Botanical contribution from the Johns Hopkins University, No. 67.

<sup>2</sup> This abstract was preprinted, without change, from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 2, no. 7, March, 1921.

study, the activity of the organisms appears clearly to be controlled by a combination of (a) salt proportions (or perhaps ion proportions), (b) total salt concentration and (c) dextrose concentration; it cannot be said to be controlled by any one or by any two of these solution conditions. When these three conditions are poorly balanced for the growth of these races, the solution may sometimes be greatly improved by altering just one of the conditions, but it is frequently necessary to alter two conditions simultaneously to obtain good physiological balance.

(7) A somewhat elaborate experimental study of the physiological properties of various combinations of the three solution conditions just mentioned (salt proportions, total salt concentration and dextrose concentration) showed one particular combination that gave by far the highest growth rates for both races, judged by dry yield. The solution representing this best combination has the following characteristics:—(a) Molecular salt proportions:  $\text{KH}_2\text{PO}_4$ , 6.0;  $\text{NH}_4\text{NO}_3$ , 1.0;  $\text{MgSO}_4$ , 1.1;  $\text{FePO}_4$ , a mere trace. (b) Total salt concentration, equivalent to a calculated osmotic value of 14.5 atmospheres. (c) Dextrose concentration, 1.0 gram-mol. per liter. The actual formula for this provisionally optimal solution follows, the partial concentration of each solute being expressed as the fraction of a gram-molecule contained in a liter:— $\text{KH}_2\text{PO}_4$ , 0.28;  $\text{NH}_4\text{NO}_3$ , 0.0462;  $\text{MgSO}_4$ , 0.0497;  $\text{FePO}_4$ , trace; dextrose, 1.0. This solution of course is not to be regarded as the established absolute optimum for these fungus races, since the experimental tests of the present study were very far from being logically complete, but it gave a much higher dry yield than did any of the other 94 solutions tested. Of course it is thus provisionally optimal only for the conditions of temperature (25–26°C.), oxygen, etc., that were here employed, and for absence of light, since all of these cultures were carried out in darkness. The oxygen supply, not artificially controlled, was unmeasured but it may safely be considered as having been practically uniform for all comparative tests.—That this provisionally optimal solution might be markedly improved if still other combinations of the three controlling solution conditions were employed is of course possible. It may be said, however, that given the salts, salt proportions and total-salt concentration of this solution, it seems improbable that any very marked improvement in physiological balance might be attained through alterations in the dextrose concentration. Nor does it appear probable that great improvement might be attained if the total-salt concentration and dextrose concentration were to remain unaltered while these salts were employed in other sets of proportions. From the evidence at hand it does appear probable, however, that a greater growth of these two *Rhizopus* races might be obtained by maintaining the same dextrose concentration and the same proportions of the ions present in this solution, but increasing the partial concentrations of the K-ions and  $\text{PO}_4$ -ions. It appears, however, that this would be impossible with the salts employed in this solution, since any increase in the total-salt concentration must cause considerable precipitation.

(8) Of course the two *Rhizopus* races dealt with in this paper are well known (from the pioneer work of Blakeslee) to be markedly different physiologically, in that they always conjugate so that one race is to be regarded as definitely female while the other is male. Although Blakeslee has suggested that this well-established physiological difference between the two races may be concomitant with differences in vegetative vigor, such differences seem not to have been definitely established hitherto. To inquire into this matter was one of the chief aims of this study, and two very pronounced physiological differences have now been established, besides the difference shown by conjugation. A third such difference is merely suggested by the present work. These differences are characterized below.

(a) Comparing the physiological characteristics of the two races by means of the criterion of dry yield alone, all the solutions tested that contained dextrose as the

carbon compound agreed in showing no definite difference. While the male and female cultures in the same solution did not nearly always give the same yields, yet there was no constant or consistent difference observed in this regard. On the other hand a clear difference between the corresponding yields of the two races appeared in the series of cultures employing glycerine as the organic compound. The evidence in this regard is not at all complete, but these glycerine cultures did uniformly give a markedly *higher* yield with the *male* than that obtained with the female race. With those particular complexes of environmental conditions (under which the yields of both races were of only medium magnitude) the male race appears to be considerably more vigorous than the female, on the basis of dry-yield production.

(b) With solutions containing dextrose as the organic substance, in all cases where yields were medium or high, another and a very striking difference between the activities of the two races was established in this study. In such cases the male race uniformly showed a much more pronounced production of sporangia than did the female, for the time periods and other conditions of these tests. Conversely, in all these cultures the female race appeared more vigorous than the male when the production of ordinary mycelium was taken as a criterion. In short, with these solutions, sporangia production is more vigorous in the male race, while mycelial growth is more vigorous in the female race (see fig. 1).

(c) An incomplete study of the influence of temperature on the comparative activities of these two races, with a single set of solution conditions, furnished the suggestion of the third difference mentioned above. On the nutrient agar used, the male race showed some growth at a temperature of from 30.5° to 31°C., while corresponding cultures of the female race showed no observable growth in the same temperature chamber and for the same time period. It is thus suggested that the maximal temperature for mycelial enlargement on this agar may be somewhat lower for the female race than for the male. Spores of the female race were not killed, however, by a 72-hour exposure to the temperature just mentioned.

(9) For the nutrient agar used in the temperature tests and for the temperatures employed, the optimal temperature for the mycelial enlargement of the male race appears to be between 25° and 28°C., while that for the female race seems to be lower, at least for the longer periods tested. As has been indicated (8 c), the maximal temperature for growth appeared to be below 31°C. for the female and above that temperature for the male race. Neither race made any growth, in these tests, with a temperature of 36°-37°C., and the minimum for both lay below 13°C.

## INTRODUCTION

It has been shown by Blakeslee<sup>3</sup> that there are two sexual races of the fungus *Rhizopus nigricans*, designated by him as *minus* and *plus*, or as male<sup>4</sup> and female.<sup>5</sup> To the author's knowledge, there has not as yet been recorded any definite morphological difference between the two races. They do show at least one clear physiological difference, however, for zygotes were formed in Blakeslee's experiments only when the conjugating hyphae differed as to race. Zygotes were never produced from two hyphae of the same race.

<sup>3</sup> Blakeslee, A. F. Sexual reproduction in the Mucorineae. Proc. Amer. Acad. Sci. **40**: 205-319. 1904.

<sup>4</sup> Blakeslee, A. F. Sexual reactions between hermaphroditic and dioecious mucors. Biol. Bull. **29**: 87-103. 1915.

<sup>5</sup> The terms male and female are generally used throughout this paper rather than *minus* and *plus*, although Blakeslee does not hold that there is convincing evidence that the minus is really male. See: Blakeslee, A. F. Sexuality in mucors. Science **51**: 375-382, 403-409. 1920.

The same author (1904) states that in those heterothallic (dioecious) fungi in which vegetative differences between the two races have been observed, the *plus* race is generally the more vigorous of the two. Although no difference was observed in the vegetative vigor or the luxuriance of growth of the two races of *Rhizopus nigricans*, they were designated as *plus* and *minus* because of their capacity for sexual response and because they form imperfect hybrids with other dioecious species the races of which do differ in vegetative vigor.

The study reported in this paper was undertaken to investigate the physiological characteristics of the two races, especially as regards their responses to nutritional conditions. Under the different nutritional, temperature, etc., conditions of these experiments, it was found that certain hitherto unrecorded differences in physiological activity are demonstrable.

This study was made with the aid of the Alice Freeman Palmer Fellowship, of Wellesley College, during the period from November, 1916, to August, 1917. The experimentation was carried on in the Botanical Laboratory and in the Laboratory of Plant Physiology, of the Johns Hopkins University. The thanks of the writer are due to that University for a Fellowship by Courtesy, by which the general facilities of the institution were freely placed at her disposal. Acknowledgment for much valued aid in planning the study and in carrying out the experimentation, as well as in the interpretation of the results obtained, are gladly made to Professor Burton E. Livingston, Professor Duncan S. Johnson, and Dr. H. E. Pulling. In the preparation of this paper for publication the writer has received much editorial help from Professor Livingston.

#### THE FUNGUS MATERIAL USED

Dried cultures of his two races of *Rhizopus nigricans* were kindly supplied by Dr. Blakeslee, from which preliminary cultures for the present study were made on agar plates. Stock cultures in liquid media were started by making transfers from these plates to a solution containing  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4$  and dextrose (in volume molecular partial concentrations of 0.0288, 0.0156, 0.010 and 0.005 respectively), with a trace of  $\text{FePO}_4$ . These were in loosely-covered glass cylinders (Stender dishes) 5 cm. in diameter and 3 cm. high, containing about 15 cc. of solution. Growth occurred rapidly with both races and numerous sporangia were soon formed. These liquid stock cultures were renewed from time to time as the work progressed, each renewal starting from a ripe sporangium taken from the preceding stock culture.

Since it appeared that the size of the sporangia tended to decrease slightly after several renewals, suggesting a correspondingly lowered vigor of the organism, a solution containing more dextrose (0.01 gram-mol. per liter)

was employed at infrequent intervals in the series, four times during the year. All inoculations for experimental cultures were made from the regular stock cultures, which were always renewed five or six days before the cultures of a new set were inoculated, in order that all such inoculations should be made with sporangia of approximately the same age. The two races were cultivated thus in liquid solution culture throughout the period from November 20, 1916, to July 15, 1917. At the end of that time both races appeared to have retained their vigor perfectly; under favorable conditions both produced a luxuriant growth of mycelium and formed sporangia abundantly. When transferred to agar they conjugated freely.

#### CULTURE METHODS

Inoculations were made by the transfer of a single mature sporangium from a stock culture, care being taken always to select sporangia of approximately the same size and general appearance. In most of the experiments the rate of growth was measured in terms of the dry weight produced during the culture period. In these cases 100-cc., flat-bottomed "Pyrex" glass flasks containing 50 cc. of solution were employed, with cotton plugs (see fig. 1). The mass of hyphae, including sporangia, was finally removed from the flask, thoroughly washed on a paper filter with distilled water and then dried to constant weight at a temperature of 103°–105°C. Stender dishes like those mentioned above, and others a little larger (6 cm. in diameter and 3.5 cm. high), were used as containers in some of the earlier experiments. In preparing the nutrient solutions, stock solutions of the separate constituents were first prepared, and proper amounts of these were then mixed, with addition of distilled water, to produce the required medium. Iron was supplied in all cases, by adding a few drops of a suspension of  $\text{FePO}_4$  such as was used by Tottingham<sup>6</sup> and Shive<sup>7</sup> in their nutrient media for wheat. Each solution was sterilized before use, three successive times, at day intervals, in a steam sterilizer of the "Arnold" type.

Richter suggests that the dry weights of *Aspergillus* obtained by Richards were "abnormal," because the fungi may have suffered from a lack of oxygen. Richards<sup>9</sup> used 125-cc. flasks containing 50 cc. of culture medium, the flasks being of course plugged with cotton. Richter grew *Aspergillus* in glass cylinders containing about 100 cc. of liquid medium, 1 cm. in depth with 500 cc. of air above the liquid. His cylinders were also plugged with cotton. In the present study the two *Rhizopus* races were generally grown

<sup>6</sup>Tottingham, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* **1**: 133–245. 1914.

<sup>7</sup>Shive, J. W. A study of the physico-chemical balance in nutrient media. *Physiol. Res.* **1**: 327–337. 1917.

<sup>8</sup>Richter, A. Zur Frage der chemischen Reizmittel. *Centralbl. Bact.* **II**, **7**: 417–429. 1901.

<sup>9</sup>Richards, H. M. Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. *Zeitschr. wiss. Bot.* **30**: 665–679. 1897.

in 100-cc. flasks containing 50 cc. of solution, as has been stated, but this question received some attention through a comparison between the dry yields of cultures in these flasks and the corresponding yields of similar cultures in glass cylinders 8.5 cm. high and 6 cm. in diameter, with 50 cc. of the same solution. Five cultures of the male and five of the female race, with solution A3d (see tables IV and V), were carried out for each type of container. Each cylinder was covered with a large piece of absorbent cotton, drawn down over the rim and fastened with a rubber band thus leaving a free air space in the dish above the solution. These cultures were all continued for a period of ten days, with a temperature of 25°-26°C.

For this comparison the average dry yield per culture was 234 mg. for the cylinders and 284 for the flasks, with the female race, and the corresponding yields with the male race were 152 and 278 mg. respectively. It appears that the flasks gave, in both cases, markedly better growth than did the cylinders. There seems to be no doubt that the shape of the culture vessel is of considerable importance in such experiments as these. It might be freely assumed that the differences here recorded between flask and cylinder cultures of the same race were directly related to the oxygen supply in the gas space of the vessels, but several other possible explanations are apparently just as probable. A marked difference was also observed with regard to the character of the growth shown by flasks and cylinders. In all flask cultures the mycelial mat, after reaching the surface of the liquid, soon became attached to the slanting wall above the solution by many aerial hyphae, so that the aerial mycelium was not as well supported in the cylinder as in the flask; in the cylinder the hyphae could become attached only to the vertical walls. Moreover, the rate of evaporation was higher in the cylinders, this being especially noticeable in the cylinder cultures of the male race, which gave relatively low yields. With the exception of a few cultures made at the beginning of the study, flasks like those shown in Figure 1 were employed in all the experimental cultures.

## EXPERIMENTATION

### I. GENERAL NUTRITIONAL REQUIREMENTS

In the early stages of the study it was found that both races grew well in a solution containing  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4$  and dextrose (with volume-molecular partial concentrations of 0.01144, 0.0156, 0.0150 and 0.0025 respectively), with a trace of  $\text{FePO}_4$ . In 5 or 6 days, with a temperature of 22°-24°C. a culture with this solution filled one of the small cylinders with vigorous mycelium and formed many sporangia. These preliminary experiments showed clearly that liquid media are very suitable for these organisms. Mycelial growth at first, takes place mainly within the solution, but aerial hyphae soon ascend into the space above, and the upper part of

the mycelial mat thus formed eventually becomes dark colored by the production of ripe sporangia.

Of course the dextrose used (although purified) must be considered as probably containing small traces of various inorganic salts. To determine whether the further addition of salts was necessary for good growth, three different concentrations of dextrose in distilled water were tested, with no addition excepting that of a trace of  $\text{FePO}_4$ . These three solutions had volume-molecular dextrose concentrations of 0.0025, 0.05 and 0.08, respectively. Twenty cultures, ten with the male (*minus*) and ten with the female (*plus*) race, were made with each solution. In 14 days, with a temperature of  $22^{\circ}$ – $23^{\circ}\text{C}$ . the least concentrated solution produced no visible growth, and growth in the most concentrated one was very slight. The 0.05 volume-molecular solution produced but little growth in 21 days, at a temperature of  $25^{\circ}$ – $26^{\circ}\text{C}$ . From ten duplicate flasks with this concentration of dextrose alone the average dry weight per culture was only 0.5 mg. for the male, and 0.3 mg. for the female race. It thus became clear that the purified dextrose did not contain the requisite inorganic salts in sufficient amount to give vigorous growth, and that inorganic salts must be added to these dextrose solutions if vigorous growth was to be obtained.

According to the literature, the nitrogen requirements for different fungi are frequently different, some being able to assimilate this element only from organic compounds, some utilizing ammonium salts, some thriving with a nitrogen source consisting of nothing but nitrates, and still others being reported as able to assimilate the element in its molecular state. Raulin<sup>10</sup> found that the dry weights of mycelium produced by *Aspergillus niger* in solutions containing  $\text{NH}_4\text{NO}_3$ , and in another without any nitrogenous compound, were, respectively, 18.3 g. and 0.12 g. The fungus grew just as well in solutions containing either  $\text{KNO}_3$  or ammonium tartrate as in those with  $\text{NH}_4\text{NO}_3$ . Richards, working with the same fungus, obtained a good growth with either  $\text{NH}_4\text{NO}_3$  or  $\text{KNO}_3$  as source of nitrogen. Out of nineteen species of *Mucor* and four other closely allied species, all isolated from the soil, Hagem<sup>11</sup> found that only ten were able to assimilate both nitrates and nitrites; those that could use nitrates could also use nitrites. This writer expresses the idea that the mucors assimilate nitrogen in the form  $\text{NH}_4$  and that certain species, as *Mucor stolonifer* (*Rhizopus nigricans*), cannot reduce the  $\text{NO}_3$  or  $\text{NO}_2$  to  $\text{NH}_4$ . In cultures of the mucors peptone has been used as a source of nitrogen more frequently than any inorganic salt. In such a study as the present one, however, the use of peptone as a source of nitrogen would have introduced many difficulties, partly because this substance would have supplied carbon in an uncertain way and also because it is

<sup>10</sup> Raulin, J. Études chimiques sur la végétation. Ann. Sci. Nat. Bot. V, 11: 93–299. 1869.

<sup>11</sup> Hagem, O. Untersuchungen über Norwegische Mucorineen. II. Vidensk. Selsk. Skr. (Math-Naturv. Kl.) 4: 1–152. 1910.

practically impossible to obtain peptone free from significant amounts of inorganic salts.<sup>12</sup>

The addition of 0.05 gram-mol. of  $\text{MgSO}_4$  per liter in one case, and the addition of 0.025 gram-mol. of  $\text{MgSO}_4$  and 0.018 gram-mol. of  $\text{KH}_2\text{PO}_4$  in another, to a 0.0025 volume-molecular solution of dextrose, produced but insignificant growth with either of the two races here studied, in a period of 14 days and with a temperature of  $22^\circ\text{--}23^\circ\text{C}$ . Evidently some nitrogen compound is necessary for the vegetative growth of both races.

To test the responses of these two races of *Rhizopus nigricans* to  $\text{Ca}(\text{NO}_3)_2$  and  $\text{NH}_4\text{NO}_3$ , four of Shives, 3-salt solutions were employed, together with three modifications of these. Dextrose was added in all cases. Referring to Shives' method of designation (1915, pages 340, 342), the four solutions used were R1C1, R4C1, R4C3 and R8C1, of Shive's optimal series (osmotic

TABLE I

Data for 3-salt solutions with addition of dextrose, to compare physiological values of  $\text{Ca}(\text{NO}_3)_2$  and  $\text{NH}_4\text{NO}_3$ . Total osmotic value, about 2 atm. ( $25^\circ\text{C}$ .)

SOLUTION NUMBER	PARTIAL CONCENTRATION (GRAM-MOL. PER LITER)					PHYSIOLOGICAL VALUE BY APPAR- ENT GROWTH <sup>b</sup>
	$\text{KH}_2\text{PO}_4$	$\text{Ca}(\text{NO}_3)_2$	$\text{NH}_4\text{NO}_3$	$\text{MgSO}_4$	Dextrose	
R1C1 <sup>a</sup>	0.0036	0.0026	.....	0.04	0.0025	Poor
R1c1	0.0036	.....	0.0052	0.04	0.0025	Good
R4C1	0.0144	0.0026	.....	0.025	0.0025	Poor
R4C3	0.0144	0.0078	.....	0.015	0.0025	Poor
r4c3	0.0144	.....	0.0156	0.015	0.0025	Good
R8C1	0.0288	0.0026	.....	0.005	0.0025	Poor
r8c1	0.0288	.....	0.0052	0.005	0.0025	Good

<sup>a</sup> The symbols refer to Shive's triangular diagram of salt proportions (Shive, 1915; fig. 1, p. 340). See also text below.

<sup>b</sup> No difference was noted, in growth-rate or general appearance, between the two races in this series.

equivalent, 1.75 atm.). These four solutions were selected as showing a wide range of salt proportions. The physico-chemical data for these solutions are given in table 1. It is to be remembered that a trace of  $\text{FePO}_4$  was added to every solution. Solution R1C1 has a high relative partial concentration of  $\text{MgSO}_4$  and low partial concentrations of  $\text{KH}_2\text{PO}_4$  and  $\text{Ca}(\text{NO}_3)_2$ . Solution R4C1 has medium relative partial concentrations of  $\text{KH}_2\text{PO}_4$  and  $\text{MgSO}_4$ , and a low one of  $\text{Ca}(\text{NO}_3)_2$ . Solution R8C1 has a high relative partial concentration of  $\text{KH}_2\text{PO}_4$  and low ones of  $\text{MgSO}_4$  and  $\text{Ca}(\text{NO}_3)_2$ . Finally solution R4C3 has medium partial concentrations of all three salts.

The three modified solutions here used were obtained by substituting  $\text{NH}_4\text{NO}_3$  for  $\text{Ca}(\text{NO}_3)_2$  in each of the Shive solutions R1C1, R4C3 and R8C1,

<sup>12</sup> H. A. L. Fisher, *Journal of Biological Chemistry*, 1915, 24, 1, 101. Also, *Journal of Biological Chemistry*, 1915, 24, 2, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999.

but the partial-concentration value for the ammonium salt was twice as great as the corresponding value for  $\text{Ca}(\text{NO}_3)_2$  in the unmodified solution. The physico-chemical data for these modifications are also shown in table I, the solutions being designated by the Shive method, but the letters being *lower case* in the present instance, instead of capitals.

Thus, solution r4c3 is like solution R4C3 excepting that the former contains 0.0156 gram-molecule of  $\text{NH}_4\text{NO}_3$  instead of 0.0078 gram-molecule of  $\text{Ca}(\text{NO}_3)_2$  per liter. No modification of solution R4C1 was employed, so that there were seven solutions in this series. It will be noted that the partial volume-molecular concentration of dextrose is the same for all solutions (0.0025). The calculated total osmotic values (osmotic "pressures") of the solutions are not markedly different, this value for each solution being slightly less than 2 atmospheres.<sup>13</sup> Solution r4c3 has the same salt proportions as the one employed for the continuous series of stock cultures, but the latter has a higher dextrose concentration.

Twenty cultures, ten with the male and ten with the female race, were made with each of these seven different solutions, the inoculations being all made from cultures with the salts as in solution r4c3. These cultures were continued 14 days, with a temperature of 19°–22°C. the small cylinders being employed. Dry weights were not obtained, but the differences in the amounts of growth produced with the solutions containing  $\text{NH}_4\text{NO}_3$ , on the one hand, and those containing  $\text{Ca}(\text{NO}_3)_2$ , on the other, were very striking. All cultures with  $\text{NH}_4\text{NO}_3$  produced large amounts of mycelium and many sporangia, but those with  $\text{Ca}(\text{NO}_3)_2$  showed only very slight growth, about like that obtained with dextrose,  $\text{MgSO}_4$ ,  $\text{KH}_2\text{PO}_4$  and  $\text{FePO}_4$ , without any nitrate at all. The last column of table I shows the sort of growth obtained with each of these seven solutions. No noticeable difference between the male and female races was observed.

Two possibilities may be considered in seeking an explanation of the apparent fact that  $\text{Ca}(\text{NO}_3)_2$  does not favor the development of these organisms, while  $\text{NH}_4\text{NO}_3$  is eminently favorable. (1) The nitrogen of  $\text{Ca}(\text{NO}_3)_2$  may not have been suitable or (2) the calcium introduced in this salt may have exerted a toxic or retarding influence. The relative physiological values of  $\text{NH}_4$ ,  $\text{NO}_3$  and  $\text{NO}_2$  were studied by means of solutions H, I, J, K and L, for which descriptive data are given in table III. Solution T, data for which are also given in table III, has its total salt concentrations nearly equally distributed among the three salts  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$  and  $\text{NH}_4\text{NO}_3$ ; it is like solution r4c3 (table I) excepting that it has 0.005 instead of 0.0025 gram-molecule of dextrose per liter. It is the solution that was generally employed for the stock cultures. Solutions T and H contain the same salts, but in different partial concentrations. Solutions H, I, J, K and L are alike with respect to dextrose,  $\text{MgSO}_4$  and  $\text{KH}_2\text{PO}_4$ , and differ only in

<sup>13</sup> See page 310 for the method employed in this study, for obtaining osmotic values.

their nitrogen content. Solution H contains both  $\text{NH}_4$  and  $\text{NO}_3$  in the salt  $\text{NH}_4\text{NO}_3$ . Solution I likewise has  $\text{NH}_4\text{NO}_3$ , although only one-fourth as much as solution H, and it is the only solution in the group containing Ca. Solution J contains no nitrate and all its nitrogen is in the salt  $(\text{NH}_4)_2\text{SO}_4$ . Solution K stands in antithesis to J, having no ammonium but having  $\text{NO}_3$ , as  $\text{KNO}_3$ . Solution L has  $\text{NO}_2$ , as  $\text{KNO}_2$ .

The approximate osmotic-pressure values for the solutions described in table III (and also for those to be considered later—tables V, IX and XI) have been calculated by assuming that the total osmotic value of all solutes in a solution is the sum of their individual osmotic values, each substance in the complex solution being assumed to act osmotically, just as though it alone were present. In other words, the influence of one substance in the solution upon the partial osmotic value due to another substance in the same solution is here neglected. The following method was used for calculating the osmotic values of the various salts: The number of ions assumed to be formed from each molecule was first decreased by unity; then the remainder was multiplied by the percentage of dissociation corresponding to the partial concentration considered ( $25^\circ\text{C}$ ), this percentage value being taken as *hundredths*. The product was then added to unity and the resulting sum was multiplied by the value representing the number of gram-molecules of the given solute in a liter of solution. Finally, the last-mentioned product was multiplied by 25, to give the approximate partial osmotic value in atmospheres. The constant 25 is employed as approximately representing the osmotic-pressure value (in atmospheres) of a volume-molecular solution of an undissociated and otherwise unmodified solute, at a temperature about  $25^\circ\text{C}$ .<sup>14</sup> It was assumed that  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4$ ,  $\text{KNO}_2$  and  $\text{KNO}_3$  each give 2 ions upon dissociation and that  $\text{Ca}(\text{NO}_3)_2$  and  $(\text{NH}_4)_2\text{SO}_4$  give 3 ions. The small number of ions assumed for  $\text{KH}_2\text{PO}_4$  follows the conclusions reached by Tottingham (1914). The values for the percentages of dissociation ( $25^\circ\text{C}$ ) were obtained from Jones' tables,<sup>15</sup> in all cases except that of  $\text{KH}_2\text{PO}_4$ . In the case of this salt, partial concentrations of 0.001, 0.005 and 0.01 gram-mol. per liter are considered as having dissociation constants, of 86, 80 and 79 per cent. respectively, these values being obtained from Wightman's data as given by Tottingham (1914, p. 177, footnote). For higher partial concentrations, up to 0.15 gram-mol., per liter lower percentage values are estimated by a method of extrapolation applied to Wightman's results. The percentages thus derived are shown below. For partial concentrations above 0.15 gram-molecule per liter the dissociation constant is taken as 50 per cent.

<sup>14</sup> Morse, H. N. The osmotic pressure of aqueous solutions. Carnegie Inst. Washington Publ. 198. 1914.  
<sup>15</sup> Jones, H. C. The electrical conductivity, dissociation, and temperature coefficients of conductivity of electrolytes in aqueous solution. Part I. A series of 100 salts and organic acids. Carnegie Inst. Washington Publ. 17. 1912.

	GRAM-MOL. PER LITER							
	0.005	0.01	0.02	0.025	0.03	0.06	0.10	0.15
Percentage of dissociation. ....	80	79	77	76	75	69	61	51

For dextrose and glycerine which are considered as undissociated and otherwise unmodified in these solutions, the number of gram-molecules per liter is simply multiplied by 25 to give the calculated partial osmotic value. Table II shows how the total osmotic values of the solutions here used have been determined, solution H being used as an illustration of the general procedure.

Twelve cultures were made with each of solutions, H, I, J, K and L, six with the male and six with the female race. Those with solutions H, I, J and K were continued 11 days with a temperature of 23°–25°C. A second

TABLE II

*Data showing chemical and physical features of solution H, to illustrate method used for calculating approximate osmotic values.*

SOLUTE	PARTIAL CONCENTRATION, GRAM-MOL. PER LITER	ASSUMED AVERAGE NUMBER OF IONS FORMED	PERCENTAGE OF DISSOCIATION	OSMOTIC VALUE, <sup>a</sup> ATMOSPHERES
KH <sub>2</sub> PO <sub>4</sub> .....	0.0036	2	83	0.165
NH <sub>4</sub> NO <sub>3</sub> .....	0.0416	2	88	1.955
MgSO <sub>4</sub> .....	0.005	2	74	0.2175
Total salts.....				2.33
Dextrose.....	0.005	Undissociated		0.125
Total solutes.....				2.46

<sup>a</sup> The osmotic value for each salt was obtained by the method described on page 13. For 0.0036 vol.-mol. KH<sub>2</sub>PO<sub>4</sub>, this value is derived as follows:  $V = 25 (0.0036) [1 + 0.83(2 - 1)]$ .

set of cultures with solutions H and K, and a set with solution L, were continued 21 days with a temperature of 25°–26°C. The average dry yield per culture, for each of these groups of six cultures, is given in the last two columns of table III.

The three solutions with NH<sub>4</sub> (H, I and J) gave a very excellent growth of both races in 11 days with the lower temperature. Solution K, with nitrogen present only in the form KNO<sub>3</sub>, gave only a very slight growth, and this was no better with the higher temperature and longer period. Solution L, with nitrogen supplied as KNO<sub>2</sub> gave but very little growth with the higher temperature and longer period. From these results it appears that neither of these organisms is able to assimilate nitrogen from NO<sub>3</sub> or NO<sub>2</sub> when one of these atomic groups is the only source of the element, and that they can assimilate this element from solutions containing NH<sub>4</sub>. They make excellent growth when NH<sub>4</sub> is supplied, either with or without calcium. For the solutions employed in this series, it appears that calcium is not injurious.

nor is an appreciable amount of it apparently essential for the active growth of these two races. Of course some calcium must have been present in the solutions tested, although no calcium compound was purposely added. Pfeffer<sup>16</sup> states that this element is not necessary for the growth of fungi in general. It is especially to be noted that  $(\text{NH}_4)_2\text{SO}_4$  appears to be just as favorable for growth as  $\text{NH}_4\text{NO}_3$ .

No clearly indicated differences between the two races are apparent in these results (table III); the fact that the male race produced a measurable yield in solutions L and K, while the female race failed to do so, is not to be emphasized; for the yields shown, a difference of 1 mg. is practically negligible in such comparisons as these.

TABLE III

Data for cultures to compare the physiological values of  $\text{NH}_4$ ,  $\text{NO}_3$  and  $\text{NO}_2$ . Each dry yield is the average from a set of six similar cultures.

SOLUTION NUMBER	PARTIAL CONCENTRATION (GRAM-MOL. PER LITER)								APPROXIMATE TOTAL OSMOTIC VALUE DUE TO		MEAN TEMPERATURE	CULTURE PERIOD	DRY YIELD	
	$\text{KH}_2\text{PO}_4$	$\text{NH}_4\text{NO}_3$	$\text{MgSO}_4$	$\text{Ca}(\text{NO}_3)_2$	$\text{KNO}_3$	$\text{KNO}_2$	$(\text{NH}_4)_2\text{SO}_4$	Dextrose	Salts alone	Salts and dextrose			Male race	Female race
									atm.	atm.			mg.	mg.
H	0.0036	0.0416	0.005					0.005	2.33	2.46	24	11	11.2	10.8
											26	21	7.6	7.2
I	0.0036	0.0104	0.005	0.0104				0.005	1.62	1.75	24	11	10.0	10.3
J	0.0036		0.005				0.0208	0.005	1.82	1.94	24	11	11.5	10.1
K	0.0036		0.005		0.0416			0.005	2.31	2.44	24	11	0.3	0.3
											26	21	0.2	0.0
L	0.0036		0.005			0.0416		0.005	2.31	2.44	26	21	1.5	0.0
T	0.0144	0.0156	0.015					0.005	1.99	2.12				

The preceding paragraphs present clear evidence (1) that these two races of *Rhizopus* may be readily grown to maturity in liquid media, (2) that dextrose is a satisfactory source of carbon, (3) that inorganic salts must be added to the medium when purified dextrose is so used, (4) that  $\text{NH}_4$  is an excellent source of nitrogen, (5) that  $\text{NO}_3$  and  $\text{NO}_2$  cannot serve as sources of this element, at least under the conditions here tested, and (6) that the element calcium is not essential for the vigorous growth of these races. Much of the succeeding experimentation dealt with the question as to how the salts  $\text{KH}_2\text{PO}_4$  and  $\text{MgSO}_4$  and the ions  $\text{NH}_4$  and  $\text{NO}_3$  should be combined with dextrose to give a solution that would be most favorable to growth.

<sup>16</sup> Pfeffer, W. The physiology of plants. Translated by A. J. Ewart. University Press, Oxford, 1900, Vol. 1, p. 432.

II. COMPARATIVE STUDY OF NUTRITIONAL VALUES OF SOLUTIONS HAVING DIFFERENT COMBINATIONS OF (1) SALT PROPORTIONS, (2) PARTIAL CONCENTRATION OF TOTAL SALTS AND (3) PARTIAL CONCENTRATION OF DEXTROSE.

The environmental conditions within a liquid medium comprise, in general, besides light, temperature and gas exchange, two features of the solution: (1) the chemical nature of the solutes and their proportions, and (2) the total concentration of the solution as this may act osmotically to influence water intake by the organism. The chemical features of the solution are (a) the nature of the solutes used and (b) their individual partial concentrations, but the latter may be stated in terms of a set of relative solute proportions. For the solutions dealt with in this section, the solutes used are  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4$  and dextrose. Their individual partial concentrations will be given in each case, but it will be convenient to deal with the three salts as a group, separately from the organic substance, and the relative partial concentrations of the salts will be considered in each case, as a set of molecular salt proportions. Also, the total concentration due to salts will be given, in each case, in terms of a calculated osmotic value stated in atmospheres of pressure. This value will be termed the *total-salt concentration*. The *dextrose concentration* in the solution will also be given as a calculated osmotic value. Finally, the total concentration of the solution will be stated in terms of atmospheres of pressure, being the sum of the *total-salt concentration* and the *dextrose concentration* (see table II). Since this last feature of any solution is merely the sum of the values for two other features, it does not need to be stated in characterizing a solution. A complete characterization of any of the solutions here considered is attained by stating (1) the salt proportions (it being understood that the three salts  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$  and  $\text{MgSO}_4$  are always the ones present and that they are always mentioned in this order), (2) the total salt concentration, and (3) the dextrose concentration. For convenience, the different sets of salt proportions used will be indicated by capital letters (A, B, etc.), the different total-salt-concentration values will be indicated by Arabic numbers (1, 2, etc.), and the different dextrose concentrations will be indicated by lower case letters (a, b, etc.). Thus, any solution may be definitely described by a combination of these three kinds of symbols (as A1b, B3d, etc.).

The individual partial concentration of each salt and that of dextrose will be stated in terms of the number of gram-molecules present in a liter of solution. These concentration values are therefore the volume-molecular partial concentrations of the various solutes. The salt proportions are the *relative* volume-molecular partial concentrations of the respective salts; they show the relative numbers of the different kinds of salt molecules present in any quantity of the solution.

Various combinations of salt proportions, total-salt concentration and dextrose concentration were tested, to determine the relative physiological values of these combinations for the two *Rhizopus* races, and a study was made of the different responses obtained with the different solutions. Three series of cultures were carried out in this connection, which will be taken up in order.

*Series I. Solutions in which the three salts occur approximately in the molecular proportions of 6:1:1, 1:10:1 or 1:1:10, the total-salt concentration, having a calculated osmotic value between 1 and 12 atmospheres of osmotic pressure, and the dextrose concentration being 0.0005, 0.005, 0.05, 0.5, 1.0 or 2.0 gram-molecules per liter.*

In series I, three different sets of salt proportions were employed (designated A, B and C), each with three different total-salt concentrations (designated 1, 2 and 3), and dextrose was used with six different partial concen-

TABLE IV

*Volume-molecular salt concentrations and molecular salt proportions for solutions of series I.*

SOLUTION GROUP	PARTIAL CONCENTRATION (GRAM-MOL. PER LITER)			APPROXIMATE MOLECULAR SALT PROPORTIONS		
	KH <sub>2</sub> PO <sub>4</sub>	NH <sub>4</sub> NO <sub>3</sub>	MgSO <sub>4</sub>	(KH <sub>2</sub> PO <sub>4</sub> : NH <sub>4</sub> NO <sub>3</sub> : MgSO <sub>4</sub> )		
A1	0.0288	0.0052	0.005	6	:	1 : 1
A2	0.08	0.0132	0.0142	6	:	1 : 1
A3	0.144	0.026	0.025	6	:	1 : 1
B1	0.0036	0.0416	0.005	1	:	10 : 1
B2	0.009	0.1152	0.0142	1	:	10 : 1
B3	0.018	0.208	0.025	1	:	10 : 1
C1	0.0036	0.0052	0.040	1	:	1 : 10
C2	0.009	0.0132	0.124	1	:	1 : 10
C3	0.018	0.026	0.2	1	:	1 : 10

trations (designated a, b, c, d, e, and f). There were thus nine different salt combinations and each of these was employed with six different dextrose contents, so that there were fifty-four different solutions in all. The data for the nine salt combinations are given in table IV.

The three sets of salt proportions (A, B, C) are derived from Shive's scheme for dealing with salt proportions and total concentration (Shive, 1915, table II, p. 342). They correspond to Shive's sets R8C1, R1C8 and R1C1, representing the three apices of his triangular diagram. In the present series, however, Shive's Ca(NO<sub>3</sub>)<sub>2</sub> is replaced by NH<sub>4</sub>NO<sub>3</sub>, as has been indicated. For total-salt concentrations 1 and 2 of the present series, the partial concentrations for KH<sub>2</sub>PO<sub>4</sub> and MgSO<sub>4</sub> are taken directly from Shive's table (optimal and supra-optimal concentrations) and each of the partial concentrations for NH<sub>4</sub>NO<sub>3</sub> are taken as double the corresponding value given for Ca(NO<sub>3</sub>)<sub>2</sub> in the same table. The two lower total-salt con-

centrations (1 and 2) of the present series correspond roughly to Shive's optimal and supra-optimal total concentrations, and the individual partial salt concentrations for the third total-salt concentration (3) are each five times the corresponding value for group 1.

The molecular salt proportions are approximately calculated in the usual way, as relative values of the three partial concentrations, the lowest partial concentration being taken as unity for all solution groups in table IV. It will be seen that each of the three sets of salt proportions (A, B and C) is characterized by a relatively high partial concentration of one salt,  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$  or  $\text{MgSO}_4$ , respectively. In solution group A the value for  $\text{KH}_2\text{PO}_4$  is about 6 times that for each of the other two salts, in groups B and C the values for  $\text{NH}_4\text{NO}_3$  and  $\text{MgSO}_4$  are each about 10 times as great as for either of the other two salts.

The six different volume-molecular partial concentrations of dextrose (a, b c, d, e, f), employed with each of the nine different salt combinations, are 0.0005, 0.005, 0.05, 0.5, 1.0 and 2.0.

Table V gives the dextrose concentrations in terms of gram-molecules per liter, also the calculated osmotic values for total-salt content and for the salts and dextrose together. It will be seen that groups 1, 2 and 3 have total-salt concentrations approximately equivalent to 2, 5 and 9 atmospheres of osmotic pressure, respectively. The molecular salt proportions for the C-group in Table V are expressed in terms of the partial concentration of  $\text{NH}_4\text{NO}_3$  taken as unity, although the partial concentration of  $\text{KH}_2\text{PO}_4$  is somewhat lower (0.7).

Ten cultures, five with the male and five with the female race, were made with each of these fifty-four different solutions. In all cases excepting those of group a (with 0.0005 vol.-mol. dextrose) the culture period was 10 days, with a temperature of 25°–26°C. Growth in the cultures of group a was so very slow that the culture period for these was extended to 16 days, but the temperature was the same as that for the other groups. The average dry yield per culture, from each of the hundred and eight sets (of five cultures each) are presented in the last two columns of table V.

Most of these sets of cultures were repeated once, some of them two, three or four times, and the results from the repetitions are essentially the same. In table VI are presented the final averages for all the sets of cultures made with each of the fifty-four solutions. When more than a single set of five cultures is employed in deriving the average yield-value, the number of sets so employed is given by the numeral in parenthesis after the yield. With the exception of the cultures of group a, the culture period was 10 days in every case, but the cultures of group a were continued 16 days. Another series with the same low partial concentration of dextrose (a) was carried out, but these were continued for 26 days with temperature varying between 17° and 22°C. The average dry yields per culture for corresponding sets in

TABLE V

*Molecular salt proportions, dextrose concentration, total-salt concentration, total concentration, and average dry yields for each solution of series I. Culture period, 10 or 16 days; temperature, 25°-26°C. Each yield is the average from a set of five similar cultures.*

SOLUTION NUMBER	MOLECULAR SALT PROPORTIONS <sup>a</sup> (KH <sub>2</sub> PO <sub>4</sub> : NH <sub>4</sub> NO <sub>3</sub> : MgSO <sub>4</sub> )	DEXTROSE CONCENTRATION  <i>gram-mol. per liter</i>	APPROXIMATE <sup>b</sup> OSMOTIC VALUE DUE TO		DRY YIELD	
			Salts alone	Salts and dextrose	Male race	Female race
			<i>atm.</i>	<i>atm.</i>	<i>mg.</i>	<i>mg.</i>
A1a <sup>c</sup>	6 : 1 : 1	0.0005	1.72	1.73	0.8	0.6
A1b	6 : 1 : 1	0.005	1.72	1.85	8.4	7.5
A1c	6 : 1 : 1	0.05	1.72	2.97	47	50
A1d	6 : 1 : 1	0.5	1.72	14.22	88	80
A1e	6 : 1 : 1	1.0	1.72	26.72	77	70
A1f	6 : 1 : 1	2.0	1.72	51.72	47	57
A2a <sup>c</sup>	6 : 1 : 1	0.0005	4.50	4.51	0.6	0.6
A2b	6 : 1 : 1	0.005	4.50	4.63	9.0	8.1
A2c	6 : 1 : 1	0.05	4.50	5.75	111	90
A2d	6 : 1 : 1	0.5	4.50	17.0	143	144
A2e	6 : 1 : 1	1.0	4.50	29.50	116	151
A2f	6 : 1 : 1	2.0	4.50	54.50	141	104
A3a <sup>c</sup>	6 : 1 : 1	0.0005	7.68	7.69	0.6	0.8
A3b	6 : 1 : 1	0.005	7.68	7.80	9.0	7.8
A3c	6 : 1 : 1	0.05	7.68	8.93	114	116
A3d	6 : 1 : 1	0.5	7.68	20.18	294 <sup>d</sup>	258
A3e	6 : 1 : 1	1.0	7.68	32.68	216	265
A3f	6 : 1 : 1	2.0	7.68	57.68	108	133
B1a <sup>c</sup>	1 : 10 : 1	0.0005	2.34	2.36	1.0	0.8
B1b	1 : 10 : 1	0.005	2.34	2.46	7.8	9.0
B1c	1 : 10 : 1	0.05	2.34	3.59	55	48
B1d	1 : 10 : 1	0.5	2.34	14.84	90	101
B1e	1 : 10 : 1	1.0	2.34	27.34	106	108
B1f	1 : 10 : 1	2.0	2.34	52.34	75	80
B2a <sup>c</sup>	1 : 10 : 1	0.0005	6.21	6.22	0.6	0.6
B2b	1 : 10 : 1	0.005	6.21	6.33	11.2	9.3
B2c	1 : 10 : 1	0.05	6.21	7.46	46	45
B2d	1 : 10 : 1	0.5	6.21	18.71	76	64
B2e	1 : 10 : 1	1.0	6.21	31.21	99	98
B2f	1 : 10 : 1	2.0	6.21	56.21	70	75
B3a <sup>c</sup>	1 : 10 : 1	0.0005	11.12	11.13	0.8	0.6
B3b	1 : 10 : 1	0.005	11.12	11.25	10.1	10.8
B3c	1 : 10 : 1	0.05	11.12	12.37	56	54
B3d	1 : 10 : 1	0.5	11.12	23.62	62	69
B3e	1 : 10 : 1	1.0	11.12	36.12	91	91
B3f	1 : 10 : 1	2.0	11.12	61.12	32	69

TABLE V—Continued

SOLUTION NUMBER	MOLECULAR SALT PROPORTIONS <sup>a</sup> (KH <sub>2</sub> PO <sub>4</sub> : NH <sub>4</sub> NO <sub>3</sub> : MgSO <sub>4</sub> )	DEXTROSE CONCENTRATION  <i>gram-mol. per liter</i>	APPROXIMATE <sup>b</sup> OSMOTIC VALUE DUE TO		DRY YIELD	
			Salts alone	Salts and dextrose	Male race	Female race
			<i>atm.</i>	<i>atm.</i>	<i>mg.</i>	<i>mg.</i>
C1a <sup>c</sup>	0.7 : 1.0 : 7.7	0.0005	1.90	1.91	0.6	0.6
C1b	0.7 : 1.0 : 7.7	0.005	1.90	2.03	9.0	8.6
C1c	0.7 : 1.0 : 7.7	0.05	1.90	3.15	37	42
C1d	0.7 : 1.0 : 7.7	0.5	1.90	14.40	72	66
C1e	0.7 : 1.0 : 7.7	1.0	1.90	26.90	51	73
C1f	0.7 : 1.0 : 7.7	2.0	1.90	51.90	48	50
C2a <sup>c</sup>	0.7 : 1.0 : 9.4	0.0005	5.36	5.37	0.8	0.6
C2b	0.7 : 1.0 : 9.4	0.005	5.36	5.49	9.0	8.6
C2c	0.7 : 1.0 : 9.4	0.05	5.36	6.61	99	82
C2d	0.7 : 1.0 : 9.4	0.5	5.36	17.86	106	136
C2e	0.7 : 1.0 : 9.4	1.0	5.36	30.36	117	113
C2f	0.7 : 1.0 : 9.4	2.0	5.36	55.36	74	108
C3a <sup>c</sup>	0.7 : 1.0 : 7.7	0.0005	8.92	8.93	0.8	0.8
C3b	0.7 : 1.0 : 7.7	0.005	8.92	9.05	15.8	10.8
C3c	0.7 : 1.0 : 7.7	0.05	8.92	10.17	111	110
C3d	0.7 : 1.0 : 7.7	0.5	8.92	21.42	<b>194</b>	158
C3e	0.7 : 1.0 : 7.7	1.0	8.92	33.92	154	<b>166</b>
C3f	0.7 : 1.0 : 7.7	2.0	8.92	58.92	65	71

<sup>a</sup> See Table IV.<sup>b</sup> Osmotic values are only approximately correct; see page 13.<sup>c</sup> Cultures of group a were continued 16 days, the others only 10 days.<sup>d</sup> The maximal yield for each set of salt proportions is shown in bold-face type.<sup>e</sup> The partial concentration of NH<sub>4</sub>NO<sub>3</sub> is here taken as unity, although that of KH<sub>2</sub>PO<sub>4</sub> is lower.

the two series with the dextrose concentration a were almost equal; in no case did they differ by more than 0.3 mg.

Comparison of the average dry yields summarized in table VI brings out the following points.

(1) The maximum yield for each race with each salt combination (including three sets of salt proportions, each with three different total salt concentrations) is shown to occur with a dextrose concentration of either 0.5 or 1.0 gram-mol. per liter (indicated by d and e respectively). These maxima are shown in table VI by bold-face type. Of the eighteen cultures with each of these two dextrose concentrations, nine gave the highest yield with 1.0 vol.-mol. (e) and nine with 0.5 vol.-mol. dextrose (d). The grand maximum for the whole series of the male race, and also for the whole series of the female, occurs with solution A3d (male, 286 mg.; female, 271 mg.). Solution A3e is also shown to have given very excellent yields for both races (male, 229 mg.; female, 270 mg.). Although the evidence is not so clear, solutions

C3d and C3e must likewise be accounted as of high physiological value for both races. Solution A3d, physiologically the best balanced of the whole series judging by dry yields, is seen to be characterized by (1) a high proportion of  $\text{KH}_2\text{PO}_4$  and low proportions of the other two salts (group A), (2) a high total-salt concentration (group 3, osmotic value for salts about 9 atm.), (3) 0.5 volume-molecular dextrose concentration.

TABLE VI

*Average dry yields for cultures of series I. The numerals in parentheses, in each case, denotes the number of sets (of five cultures) from which the average is derived. Temperature, 25°-26°C. Culture period, 10 days, excepting for group a, in which case it was 16 days.*

SOLUTION NUMBER	RACE	0.0005 VOL.-VOL. DEXTROSE (a)	0.005 VOL.-VOL. DEXTROSE (b)	0.05 VOL.-VOL. DEXTROSE (c)	0.5 VOL.-VOL. DEXTROSE (d)	1.0 VOL.-VOL. DEXTROSE (e)	2.0 VOL.-VOL. DEXTROSE (f)
		mg.	mg.	mg.	mg.	mg.	mg.
A1	Male	0.8 (1)	8.4 (1)	47 (1)	88 (1)	70 (2)	47 (1)
	Female	0.6 (1)	7.5 (1)	50 (1)	80 (1)	59 (2)	57 (1)
B1	Male	1.0 (1)	9.5 (1)	56 (3)	104 (2)	109 (3)	75 (1)
	Female	0.8 (1)	9.9 (2)	56 (3)	99 (2)	104 (2)	80 (1)
C1	Male	0.6 (1)	9.0 (1)	37 (1)	72 (1)	46 (2)	48 (1)
	Female	0.6 (1)	8.6 (1)	42 (1)	66 (1)	71 (2)	50 (1)
A2	Male	0.6 (1)	9.0 (1)	111 (1)	154 (2)	146 (2)	130 (2)
	Female	0.6 (1)	8.1 (1)	90 (1)	154 (2)	159 (2)	98 (2)
B2	Male	0.6 (1)	11.2 (1)	54 (3)	84 (2)	92 (2)	70 (1)
	Female	0.6 (1)	9.3 (1)	61 (3)	72 (2)	93 (2)	75 (1)
C2	Male	0.8 (1)	9.0 (1)	99 (1)	121 (2)	118 (3)	72 (2)
	Female	0.6 (1)	8.6 (1)	82 (1)	116 (2)	115 (3)	85 (2)
A3	Male	0.6 (1)	9.0 (1)	121 (2)	286 (2)	229 (2)	139 (2)
	Female	0.8 (1)	7.8 (1)	123 (2)	271 (2)	270 (3)	150 (2)
B3	Male	0.8 (1)	10.1 (1)	59 (4)	75 (2)	88 (2)	29 (2)
	Female	0.6 (1)	10.8 (1)	53 (4)	76 (2)	86 (2)	63 (2)
C3	Male	0.8 (1)	15.8 (1)	117 (2)	191 (2)	147 (4)	73 (2)
	Female	0.8 (1)	10.8 (1)	110 (2)	165 (2)	172 (4)	71 (1)

(2) It is clear from tables V and VI that the physiological optimum for the whole series (solution A3d) is determined by the proportions of the three salts, the total-salt concentration and the dextrose concentration. Nine salt combinations were employed with each dextrose concentration. The maximal yield with each of the four higher partial dextrose concentrations (0.05, 0.5, 1.0 and 2.0 vol.-mol.) was obtained with solutions of group

A3. With the two lower dextrose concentrations the conditions for growth were apparently so unfavorable that neither the salt proportions nor the total-salt concentration influenced the yields. Solution A3d appears to combine a very excellent set of salt proportions (A3) with a very excellent partial concentration of dextrose (d), so as to give the maximum dry yield of the whole series. In considering the relation of salt proportions to dry yields, it will be noted that group A3 has a high relative partial concentration of  $\text{KH}_2\text{PO}_4$  and low ones of the other salts, while solutions C3d and C3e (which also gave very large yields) have a high proportion of  $\text{MgSO}_4$  and low ones of the other two salts. As far as salt proportions are concerned, it may be said that excellent growth was *not* obtained with solutions having a very high relative partial concentration of  $\text{NH}_4\text{NO}_3$ .

(3) The last remark suggests that it may not be the salt proportions *as such* that render solutions having a high proportion of  $\text{NH}_4\text{NO}_3$  unbalanced for excellent growth; it may be the actual partial concentration of this particular salt. This suggestion appears to be supported by the fact that the highest yields of the B-series (with high proportion of  $\text{NH}_4\text{NO}_3$ ) occurred with the lowest total-salt concentration tested (group 1). While solution B1d gave yields of 109 and 104 mg. for the two races, respectively, solution B2e gave 92 and 93 mg., and solution B3e gave even lower values, 88 and 86 mg. It is possible that this relation between yield and the actual amount of  $\text{NH}_4\text{NO}_3$  present in a volume unit of solution may be due to an accumulation of acid in the medium (possibly due to selective absorption). This question was not studied in connection with the present work, but other writers have recorded results similar to those presented here and have brought forward evidence in favor of acidity as a clue to the explanation. Medisch<sup>17</sup> cultivated *Hypocrea rufa* (an ascomycete) in a nutrient solution containing dextrose,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$  and either  $\text{NH}_4\text{NO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{NH}_4\text{Cl}$ , and found that the three last-named salts, with partial concentration of 0.1 gram-mol. per liter, retarded the growth of the fungus. Medisch obtained good growth in a similar solution in which nitrogen was supplied as in 0.1 vol-mol.  $\text{KNO}_3$ , with no ammonium salt. When  $\text{CaCO}_3$  was added to a solution containing an ammonium salt the resulting yield was twice as great as when the carbonate was not present. Tests showed that all the solutions containing an ammonium salt were strongly acid unless  $\text{CaCO}_3$  was added, and Medisch concludes that the nutrient salts are not available to the fungus in such solutions on account of the high acidity. Hagem (1910) made similar observations with regard to the accumulation of acid in mucor cultures containing the ammonium salts  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NH}_4\text{Cl}$  and  $\text{NH}_4\text{NO}_3$ , and noted that beneficial results were produced by neutralizing the acidity of the medium with  $\text{CaCO}_3$ . He observed that the fungus assumed a peculiar form when growing in an acid culture, calling this form "acid mycelium;" the

<sup>17</sup> Medisch, M. Beiträge zur Physiologie der *Hypocrea rufa*, Jahrb. wiss. Bot. 48: 591-631. 1910

hyphae walls were thicker and the branches were shorter and larger in diameter than in the usual form. Greater growth resulted with  $(\text{NH}_4)_2\text{PO}_4$  than with any of the other three ammonium salts employed by Hagem, and he considers this as due to the fact that  $\text{H}_2\text{PO}_4$  (the acid formed from  $(\text{NH}_4)_2\text{PO}_4$ ) does not readily dissociate, so that the employment of this ammonium salt produces only a comparatively slight accumulation of  $\text{H}$ -ions. He states that fungus forms that were able to reduce nitrates produced no accumulation of acid in the presence of  $\text{NH}_4\text{NO}_3$ , which suggests that such forms absorb the  $\text{NO}_3$ -ion as rapidly as they do the  $\text{NH}_4$ -ion, thus leaving the solution nearly neutral.

Steinberg,<sup>1</sup> from his study of *Aspergillus niger* grown in nutrient solutions, presented some interesting data in regard to the effect of increasing the acidity of the medium. He found that the addition of  $\text{HNO}_3$  or  $\text{HCl}$ , in certain concentrations, to Pfeffer's solution increased the growth more than 100 per cent., as measured by the dry yield. The acidity of the control cultures with Pfeffer's solution was  $\text{P}_{\text{H}}$  3-4. Apparently the mucors, *Aspergillus* and other forms studied react very differently to an increase in the acidity of the medium. The acidity of the solutions was not determined in the present study, so that further light cannot be thrown on this question here.

(4) Considering the solutions having dextrose concentrations of 0.5 and 1.0 gram-mol. per liter (d and e) together, it is interesting to note that the dry yield is progressively greater with progressively higher total-salt concentration, within the A- and C-groups (low relative partial concentrations of  $\text{NH}_4\text{NO}_3$ ) but that the dry yield is greater within the B-group (high relative partial concentration of  $\text{NH}_4\text{NO}_3$ ) for the lowest total salt content (B1). If increasing the total salt concentration of solutions of the B-group produces higher  $\text{H}$ -ion content, it may be supposed that the B2- and B3-groups give lower yields than does the B1-group on account of higher acidity in the latter case. That the highest yields for the B-group (with the lowest total-salt concentrations of that group) are markedly lower than the maximum yields for the A- and C-groups (with the highest total salt concentrations of these groups), may also be due to comparatively high  $\text{H}$ -ion concentrations in the B1-solutions, in which most of the total-salt concentration is due to  $\text{NH}_4\text{NO}_3$ . These points are set forth in table VII, which is a summary of table VI in this regard.

It is of course logically possible that still higher total concentrations than those of groups A3 and C3, and still lower ones than those of group B1, might give still higher yields, with dextrose concentration d or e, than those recorded for A3d, C3d and B1e respectively. This matter will receive further attention below.

<sup>1</sup> Steinberg, R. A. A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. 5: 449-456, 357-372. 1919.

(5) In connection with the points just emphasized it should be noted that, for dextrose concentrations of 0.5 or 1.0 gram-mol. per liter the optimal set of salt proportions is not the same for all three total-salt concentrations. For the lowest total-salt concentration (1) the set of salt proportions designated by B gave the greatest yields, while for the other two (2 and 3) the A-set of salt proportions gave highest yields. Stating this same relation in another way, for the sets of salt proportions denoted by A and C the optimal total-salt concentration is the highest of the three tested (3), but for the B-set of salt proportions the optimal total-salt concentration is the lowest of the three (1). These results furnish further evidence of the general truth of the apparently important proposition put forward by Tottingham and supported by Shive (referring to their studies of the salt nutrition of young wheat plants), to the effect that the optimal total-salt concentration is not the same for markedly different sets of salt proportions.

TABLE VII

*Summary of data (series I) on influence of salt proportions and total-salt concentration, with 0.5 or 1.0 gram-mol. of dextrose per liter (from table VI). Each yield is the average from a set of five similar cultures.*

SALT COMBINATION	AVERAGE DRY YIELD		SALT COMBINATION	AVERAGE DRY YIELD		SALT COMBINATION	DRY YIELD	
	Male race	Female race		Male race	Female race		Male race	Female race
	mg.	mg.		mg.	mg.		mg.	mg.
A1	76	72	A2	147	157	A3	<b>286</b>	<b>271</b>
B1	<b>109<sup>a</sup></b>	<b>104</b>	B2	80	76	B3	78	70
C1	57	67	C2	121	123	C3	<b>227</b>	<b>178</b>

<sup>a</sup> The maximum yield for each set of salt proportions (A, B, C) is shown in bold-face type.

(6) Turning to the influence of the partial concentration of dextrose in the medium it appears that, for every one of the salt combinations here tested, the partial dextrose concentration very definitely determines the yield for both races. As has been said, all the maxima (bold-face type in table VI) occur with solutions containing either 0.5 or 1.0 gram-mol. of dextrose per liter. Furthermore, the approximate optimum is clearly brought out in every case; volume-molecular dextrose concentrations lower than 0.5, and those higher than 1.0, exhibit yields markedly lower than the maximum.

(7) With the lowest dextrose concentration tested (0.0005 gram-mol. per liter, group a) the yields were very low in all cases and, considering the practical difficulties of completely recovering the harvest, as well as the relative inaccuracies of weighing these very small yields, they are to be regarded as sensibly equal. This means that when the supply of dextrose is very low these fungus races are not appreciably influenced by either salt proportions, or total-salt concentration, growth being very much retarded in all cases. Solutions with only 0.0005 gram-mol. of dextrose per liter (group a) produced

greater growth, however, than did dextrose solutions without the addition of any salts at all (see page 307), although the pure sugar solutions possessed much higher dextrose concentrations (0.05 and 0.08 vol.-mol.). With the next higher dextrose concentration (0.005 gram-mol. per liter, group b) the yield values are always ten or more times as great as those in group a, but the salt proportions are again without pronounced influence. With the third dextrose concentration (0.05 gram-mol. per liter, group c) and also with the optimal concentrations (groups d and e), the yields produced in the solutions with the A- and C-sets of salt proportions (low  $\text{NH}_4\text{NO}_3$ -content) are higher with greater total-salt concentration. The yields from solutions A3c and C3c are from two-and-one-half to three times as great as those from solutions A1c and C1c. With dextrose concentrations above the optimum (as shown by group f, 2.0 gram-mol. per liter), the relations of yield to salt proportions and to total-salt concentration are generally the same as those in groups d and e. With still higher dextrose concentrations these relations should progressively vanish, since all the yields must become smaller and approach zero at this end of such a series.

From the statements of the last paragraph it is clearly indicated that salt proportions, and total-salt concentration are influential upon the growth of these two races of *Rhizopus* only in the presence of a suitable concentration of dextrose; the carbohydrate supply appears to be a main limiting condition for growth with all combinations of total-salt concentration and salt proportions. It is of course possible, however, that the highest dextrose concentration (f, 2 vol.-mol.) retards growth because the osmotic value of the solution is too great, rather than because of too great a supply of sugar.

(8) The different degrees to which dextrose concentration influences the physiological values of the nine different salt combinations here tested deserves attention. The range of this influence is shown by the following comparison:—With an increase in sugar concentration from 0.0005 to 0.5 volume-molecular, the yield obtained for the male race with salt combination A1 was increased from 0.8 to 88 mg., and that obtained with salt combination A3 was increased from 0.6 to 286 mg. In both cases the higher sugar concentration was 1000 times as great as the first, but the yield was increased by a factor of only 110 ( $\frac{88}{0.8}$ ) in the first case, and by one of 476 ( $\frac{286}{0.6}$ ) in the second. With the highest total-salt concentration (3) of this particular set of salt proportions (A), the influence of multiplying the dextrose concentration by 1,000 was 4.3 times as great as the corresponding influence with the lowest total-salt concentration (1) and the same set of salt proportions (A). Other comparisons of similar nature may be made by means of the data of table VI. The outcome of such a study indicates that the physiological influence of sugar concentration, when it alone is considered as a variable, is determined by the salt combination employed. It is of course logically necessary to suppose that this influence of the dextrose

concentration would itself vanish if the salt features of the medium were such as almost to inhibit growth, as is indeed indicated by the tests in which no salts were used (see p. 307).

(9) Considering the relations between the two fungus races with regard to growth vigor, the data of table VI show that the male and female yields are approximately equal for most of the solutions.<sup>19</sup> There is apparently no marked tendency for the dry yields of one race to exceed those of the other with any concentration of dextrose, no matter what total-salt concentration and what set of salt proportions are used. In the actual optimal solution of this series (A3d) the male yield is slightly larger than the female, but the reverse is true with solution A3e, which is the second-best solution on the basis of dry yields.

(10) While a comparison of the dry yields from the fifty-four solutions of this series fails to establish any definite physiological difference between the two races, the general appearance of the cultures under discussion brought out what may be an important point in this general connection. It generally appeared that, for solutions giving high dry yields, and for the temperature conditions tested, spore production became much more pronounced in the male than in the female race, as the cultures approached maturity. Furthermore, while the male race produced many more sporangia than did the female race under the same conditions, it was just as clearly apparent that the female race produced a greater volume of mycelium than did the male. Although the dry yields from the two races are practically the same for any environmental complex, the activity of the male race soon becomes largely that of spore production, while the activity of the other consists predominantly in the formation of vegetative hyphae. In the early stages of all cultures growth activity was of course entirely directed toward the production of vegetative hyphae.

The relative-score method described by Free<sup>20</sup> was employed to compare the different rates of spore production and of vegetative growth in the cultures of the two races. For the nine different solutions with dextrose concentration of 2.0 gram-mol. per liter, the average spore-production score was 9.2 for the male and 5.0 for the female race, so that spore production was apparently nearly twice as vigorous in one case as in the other. The same general relation held for solutions having dextrose concentrations of 0.05, 0.5 and 1.0 gram-mol. per liter. The more favorable the solution was for growth, the more marked was this difference in the mode of growth of the two races. The dry yields produced with the solutions of groups B2 and B3 were always less, and a greater number of sporangia were produced, than

<sup>19</sup> A difference of 10 per cent. or less is considered as negligible, because the dry yields from the same solution with the same race in different culture series showed as much variation as 10 per cent.

<sup>20</sup> Free, E. E. A relative score method of recording comparisons of plant condition and other unmeasured characters. *Plant World* **18**: 249-256, 1915.

with the solutions of groups A2, A3, C2 and C3, the same concentration of dextrose being employed. As to growth, the male and female races were more alike in solutions of the two former groups than in those of the latter groups. Cultures of the two races with solutions A3d and C3d showed a very striking difference in appearance after a growth period of 8 days. The male cultures appeared black with ripe sporangia while the female cultures showed merely masses of white, fluffy mycelium, with mature sporangia only in the neck of the flask. Thus, in their mode of growth, the male and female races respond very differently to the same external conditions and furnish evidence of a very definite physiological difference between the two races, although this difference is not to be detected by a comparison of dry yields. This difference in the mode of growth of the two races has not, to



FIG. 1. Cultures of male (♂) and female (♀) races of *Rhizopus nigricans*, grown 10 days in solution A5d (see table IX). Note the greater development of sporangia (indicated by dark color) by the male race. The conspicuous white reflection is of sky through greenhouse roof.

the writer's knowledge, been hitherto established, although it can be observed to a slight extent in cultures on agar slants. Blakeslee, at one time, seems to have suspected the presence of such a physiological difference, but his cultures failed to establish it clearly, and he remarks (1904): "The only difference, therefore, by which at present the strains of *Rhizopus* can be distinguished lies in their sexual action when grown in contact." The difference in appearance between male and female cultures in solutions of the same kind and with the same temperature, etc., conditions, is illustrated by the photographs of figure 1.

Nutrient solutions that were unfavorable to general development rendered the two races more nearly alike in their manner of growth, yet no one of the solutions here tested was effective to *reverse* this observed difference between

the races. It is of course quite possible that a reversal might be accomplished with a proper combination of environmental conditions.

*Series II.* Solutions in which the three salts occur approximately in the molecular proportions of about 360:1:1, 1:600:1 or 1:1:570, the total salt concentration being approximately equivalent to 6.8, 11.8 or 8.8 atmospheres of osmotic pressure, and the dextrose concentration being about 0.005, 0.05 or 0.5 gram-mol. per liter.

In the series of cultures just considered (series I) the three sets of molecular salt proportions for the A3-, B3- and C3-groups were approximately 6:1:1, 1:10:1 and 1:1:10 respectively, so that each salt occurred in the entire series in two partial concentrations having a ratio in no case higher than 10:1. Also, in the series about to be considered each salt was employed in only two partial concentrations but the ratio of the higher to the lower of these concentrations was much greater than in series I; in no case was it less

TABLE VIII

*Volume-molecular salt concentrations and molecular salt proportions for solution groups A3, B3, C3, E3, F3, and G3, of series I and II. (The data for the first three are repeated from table IV, for comparison.)*

SOLUTION GROUP	PARTIAL CONCENTRATION (GRAM-MOL. PER LITER)			APPROXIMATE MOLECULAR SALT PROPORTIONS		
	KH <sub>2</sub> PO <sub>4</sub>	NH <sub>4</sub> NO <sub>3</sub>	MgSO <sub>4</sub>	(KH <sub>2</sub> PO <sub>4</sub> : NH <sub>4</sub> NO <sub>3</sub> : MgSO <sub>4</sub> )		
A3	0.144	0.026	0.025	6	:	1 : 1
B3	0.018	0.208	0.025	1	:	10 : 1
C3	0.018	0.026	0.20	1	:	1 : 10
E3	0.18	0.00052	0.0005	360	:	1 : 1
F3	0.00036	0.26	0.0005	1	:	600 : 1
G3	0.00036	0.00052	0.25	1	:	1 : 570

than 360:1. The three new sets of salt proportions introduced in this series are designated as E3, F3 and G3; they were derived from the lowest volume-molecular concentration of each salt employed in series I. The lowest concentration of KH<sub>2</sub>PO<sub>4</sub>, NH<sub>4</sub>NO<sub>3</sub> and MgSO<sub>4</sub> in series I were respectively 0.0036, 0.0052 and 0.005 gram-mol. per liter, while the corresponding values for series II were 0.00036, 0.00052 and 0.0005. Table VIII presents the partial concentration of each salt for each of these new solution groups and also shows the molecular proportions of the three salts in each case. The present series included three different partial concentrations of dextrose for each set of salt proportions, these dextrose concentrations being those heretofore designated as b, c and d (table III); i.e., 0.005, 0.05 and 0.5 gram-mol. per liter, respectively.

The nine different solutions comprising this series (each of the three sets of salt proportions being employed with three different partial concentrations of dextrose) were tested with each of the two races, five flasks of each solu-

tion being employed for the male and five for the female race. The yields obtained, and the other data not yet given for this series, are shown in table IX.

The dry yields shown in table IX are similar to the yields recorded for groups 1b and 2b in table VI and they are comparable to those for group a, in the general uniformity of the yields of both races in nine different solutions. The solutions of groups E, F and G with the two higher partial concentrations of dextrose (0.05 and 0.5 gram-mol. per liter) yielded very different results from those obtained with these same concentrations of

TABLE IX

*Molecular salt proportion, dextrose concentration, total-salt concentration, total concentration, and average dry yields for each solution of series II. Culture period, 10 or 17 days<sup>a</sup>; temperature, 25°–26°C. Each yield is the average from a set of five similar cultures.*

SOLUTION NUMBER	MOLECULAR SALT PROPORTIONS <sup>b</sup> (KH <sub>2</sub> PO <sub>4</sub> : NH <sub>4</sub> NO <sub>3</sub> : MgSO <sub>4</sub> )	DEXTROSE CONCENTRATION  gram-mol. per liter	APPROXIMATE <sup>c</sup> OSMOTIC VALUE DUE TO		DRY YIELD	
			Salts alone	Salts and dextrose	Male race	Female race
			atm.	atm.	mg.	mg.
E3b	360 : 1 : 1	0.005	6.80	6.93	4.2 <sup>d</sup>	4.2 <sup>d</sup>
E3c	360 : 1 : 1	0.05	6.80	8.05	5.8	6.2
E3d	360 : 1 : 1	0.5	6.80	19.30	5.1	7.2
F3b	1 : 600 : 1	0.005	11.83	11.95	7.6 <sup>d</sup>	7.6 <sup>d</sup>
F3c	1 : 600 : 1	0.05	11.83	13.07	7.8	6.2
F3d	1 : 600 : 1	0.5	11.83	24.32	9.0	9.2
G3b	1 : 1 : 570	0.005	8.76	8.89	7.6 <sup>d</sup>	7.6 <sup>d</sup>
G3c	1 : 1 : 570	0.05	8.76	10.01	7.8	6.2
G3d	1 : 1 : 570	0.5	8.76	21.26	9.0	9.2

<sup>a</sup> The culture period for the b and c groups was 17 days, that for the d group was only 10 days.

<sup>b</sup> See table IV, p. 314.

<sup>c</sup> Osmotic values are only approximate, see page 13.

<sup>d</sup> For the solutions with the lowest dextrose concentration (group b) the yield values were so low that no attempt was made to obtain the male and female yields separately; they were determined together as a combined yield and the average given are alike for the two races, each being half of the combined value.

dextrose and the salt proportions of groups A, B and C. It is clear that the combinations of salt proportions and total-salt concentration tested with groups E, F and G were definitely unsuitable for satisfactory growth with any dextrose concentration employed. From the data of series I it appears that salt proportions, total-salt concentration and dextrose concentration are three conditions that act together to determine the activities of these fungus races, under the given oxygen, light, temperature, etc., conditions employed in this study; each one of these three conditions must be properly adjusted if satisfactory growth of either race is to be obtained. If one or

two of the three conditions is not suitable for rapid growth activity it makes no difference what may be the values of the other one or two, poor growth results in every case where *all three* are not properly adjusted and combined.

On the basis of the dry yields, the two races produced practically equal growths in all nine solutions, but there was a striking difference as regards appearance and general manner of growth in some of the cultures. Aerial mycelium and sporangia were formed in all the cultures with solutions of groups E and G. The usual tuft of submerged hyphae was produced and a thin hyphal network formed on the surface of the liquid, while the aerial mycelium, including the sporangiophores, developed above. In about half of the cultures of group F the mycelium remained entirely submerged and, consequently, no sporangia were produced. In other cultures of group F development occurred as in groups E and G. In cultures of the F-group the submerged portion of the mycelium presented a very different appearance from the aerial growth or from the submerged mycelium in the cultures of groups E and G. This submerged mycelium of the F-cultures was composed of short, stout, knobby, thick-walled filaments, markedly different in appearance from the typical long, straight, slender, thin-walled hyphae that were formed in the solutions of all the other groups. When any of these thick-walled hyphae reached the surface of the liquid, as they occasionally did when the cultures were continued for a period of 17 days, they grew out into the air, forming typical slender hyphae.

This peculiar submerged mycelium, encountered only with solutions of group F, appears to correspond to Hagem's "acid mycelium" (see page 319). If this type of growth is conditioned by high concentration of H-ions, as Hagem implies, it might indeed be looked for with the F-solutions, since these had relatively high partial concentrations of  $\text{NH}_4\text{NO}_3$  and might therefore be expected to become more strongly acid than would solutions of most of the other groups.

Since this type of growth in the solutions of group F constituted the only peculiar structural response met with in this whole study, a special series of cultures was planned to inquire into the possibility of producing some corresponding physiological peculiarity that might alter the sexual characters of one or both of the races. The organisms were grown for a long period in solutions of groups E, F and G. This experiment was begun Feb. 15, when cultures of the male and female race were made with solutions E3b, F3b and G3b. On March 9 transfers of sporangia were made from each of these cultures to new solutions of the same character. Similar transfers were again made on March 20, but this time the new solutions used differed from the old in that their dextrose content was higher; these were E3c, F3c and G3c (containing 0.05 gram-mol. of dextrose per liter). Transfers were subsequently made to new c-solutions, at irregular intervals of about two or three weeks (without altering the nature of the solutions further) until

June 26, three cultures of each race being made for each solution. The last set of these cultures, terminating June 26, showed the usual growth with solutions E3c and G3c, while solution F3c produced no aerial hyphae and exhibited the peculiar "acid" submerged mycelium described above.

These cultures were then tested for conjugation, on agar plates. Three kinds of agar were used, made up with solutions E3c, F3c and G3c, respectively. Male and female stocks from solution F3c failed to produce zygotes when grown together on an agar plate containing solution F3c, although a poor growth occurred (the mycelium being the typical and not the "acid" form) and a few sporangia were formed by both races. On the other hand, the two stocks from solution G3c produce a few zygotes when grown together on F3c-agar, and more zygotes on G3c-agar. The two stocks from solution E3c gave many zygotes when grown together on E3c-agar. These tests show that the stocks from solutions E3c and G3c had retained their power of conjugation, while those from solution F3c failed to show their original power to conjugate when grown together on F3c-agar, which was probably due to the fact that the races had been somewhat weakened by the long culture period (4 months) in a solution which was unfavorable for their development. The female stock from solution F3c did not conjugate with the female stock from solution G3c when they were grown together on F3c-agar, nor did it conjugate with the female stock from solution E3c when grown with the latter on E3c-agar. Likewise, the male stock from solution F3c failed to conjugate with the same race from solution G3c when they were grown together on G3c-agar. These tests, although very incomplete, fail to show evidence of any fundamental change in the conjugating power in either of the two races from the cultures with solution F3c, although both races were markedly weak vegetatively and had been producing the peculiar "acid" form of submerged hyphae.

*Series III. Solutions in which the three salts occur in the volume-molecular proportions used in series I, but with total-salt concentrations having calculated osmotic values between 1 and 16 atmospheres of osmotic pressure and with a dextrose concentration of either 0.5 or 1.0 gram-mol. per liter.*

From the data of series II and III it is evident (tables VI and IX) that the highest dry yields thus far obtained were with solutions A3d, A3e, B1d, B1e, C3d, and C3e. The present series was planned to determine whether still higher total-salt concentrations than those of groups A3 and C3, and still lower ones than those of group B1, when employed with 0.5 or 1.0 volume-molecular dextrose, might give higher yields than any yet recorded with these three sets of salt proportions. The solutions used in series III are characterized, as far as their salt proportions and total-salt concentrations are concerned, by the data of table X.

In this series the letters, A, B, and C, have the same meanings as in series I (table IV). The solutions indicated by T have been characterized (p. 309

and table III). The molecular proportions for this group are approximately:  $\text{KH}_2\text{PO}_4$ , 0.7:  $\text{NH}_4\text{NO}_3$ , 1.0:  $\text{MgSO}_4$ , 1.0.) The total salt concentrations for series III are indicated by numerals as before, 1, 2 and 3 having the same meanings as previously. Concentrations  $\frac{1}{2}$ , 4, 5 and 6, which have not been encountered before, have values respectively about 0.2, 2.5, 3.0 and 3.5 times as great as the value for concentration 2. Only two concentrations of dextrose were employed in series III, 0.5 and 1.0 gram-mol. per liter, indicated, as before, by the small letters d and e.

The solutions selected for this series were those logical possibilities offered by the general scheme of concentrations, that seemed to give promise of

TABLE X

*Volume-molecular salt concentrations and molecular salt proportions for solutions of series III. Data for solutions A2, A3, B1, C2 and C3 are repeated from table IV, for comparison.)*

SOLUTION GROUP	PARTIAL CONCENTRATION (GRAM-MOL. PER LITER)			APPROXIMATE MOLECULAR SALT PROPORTIONS ( $\text{KH}_2\text{PO}_4$ : $\text{NH}_4\text{NO}_3$ : $\text{MgSO}_4$ )		
	$\text{KH}_2\text{PO}_4$	$\text{NH}_4\text{NO}_3$	$\text{MgSO}_4$			
A2	0.08	0.0132	0.0142	6	:	1 : 1
A3	0.144	0.026	0.025	6	:	1 : 1
A4	0.2	0.033	0.0355	6	:	1 : 1
A5	0.24	0.0396	0.0426	6	:	1 : 1
A6	0.28	0.0462	0.0497	6	:	1 : 1
B1	0.0036	0.0416	0.005	1	:	10 : 1
$B\frac{1}{2}$	0.002	0.0238	0.003	1	:	10 : 1
C2	0.009	0.0132	0.124	1	:	1 : 10
C3	0.018	0.026	0.20	1	:	1 : 10
C4	0.0225	0.033	0.310	1	:	1 : 10
C5	0.027	0.0396	0.372	1	:	1 : 10
T2	0.0303	0.0428	0.0475	0.7	:	1 : 1
T4	0.075	0.110	0.1182	0.7	:	1 : 1

producing more vigorous growth than had been obtained with the best solutions of series I. Ten cultures were made with each solution, five with the male and five with the female race. All cultures were continued for a period of 10 days with a temperature of 25°–26°C. The molecular salt proportions, total-salt concentration, dextrose concentration and total concentration, as well as the average dry yields obtained, for each solution tested, are set forth in table XI, following the general notation used in tables V and IX.

The results shown in table XI may be discussed under the four headings given below.

(1) *Influence of total-salt concentration.* Of the solutions tested that had salt proportions of group C, no increase in yield was obtained with higher

total-salt concentration than that numbered 3. The highest yield for both races was obtained with solution C3d, the male yield being 191 mg. and the female 165 mg.

With the solutions having the salt proportions of group A, however, the dry yields were markedly larger with higher total-salt concentration than

TABLE XI

*Molecular salt proportions, dextrose concentration, total-salt concentration, and average dry yields for each solution of series III. Culture period, 10 days; temperature, 25-26°C. Each yield is the average from a set of five similar cultures. (Data for solutions A3d, A3e, B1d, C3d, and C3e are repeated from tables V and VI, for comparison.)*

SOLUTION NUMBER	MOLECULAR SALT PROPORTIONS <sup>a</sup> (KH <sub>2</sub> PO <sub>4</sub> : NH <sub>4</sub> NO <sub>3</sub> : MgSO <sub>4</sub> )	DEXTROSE CONCEN- TRATION  <i>gram-mol. per liter</i>	APPROXIMATE OSMOTIC <sup>b</sup> VALUE DUE TO		DRY YIELD	
			Salts alone	Salts and dextrose	Male race	Female race
A2d	6 : 1 : 1	0.5	4.50	17.0	154	154
A3d	6 : 1 : 1	0.5	7.68	20.18	286	271
A3e	6 : 1 : 1	1.0	7.68	32.68	229	270
A4d	6 : 1 : 1	0.5	10.40	22.90	327	336
A5d	6 : 1 : 1	0.5	12.47	24.97	318	330
A5e	6 : 1 : 1	1.0	12.47	37.47	314	340
A6e	6 : 1 : 1	1.0	14.50	39.50	{ 380 <b>391<sup>d</sup></b>	<b>420<sup>c</sup></b> 408 <sup>c</sup>
B1d	1 : 10 : 1	0.5	2.46	14.96	90	<b>89</b>
B $\frac{1}{2}$ d	1 : 10 : 1	0.5	1.36	13.86	<b>98</b>	88
C2d	1 : 1 : 10	0.5	5.36	17.86	121	116
C3d	1 : 1 : 10	0.5	8.92	21.42	<b>191</b>	<b>165</b>
C3e	1 : 1 : 10	1.0	9.11	33.92	147	162
C4d	1 : 1 : 10	0.5	12.94	25.44	153	132
C4e	1 : 1 : 10	1.0	12.94	37.94	179	161
C5d	1 : 1 : 10	0.5	15.34	27.84	141	125
C5e	1 : 1 : 10	1.0	15.34	40.34	151	154
T2d	0.7 : 1 : 1	0.5	5.06	17.56	155	157
T4d	0.7 : 1 : 1	0.5	12.28	24.78	<b>207</b>	<b>203</b>

<sup>a</sup> See table X.

<sup>b</sup> Osmotic values are only approximate; see page 310.

<sup>c</sup> Average from two sets of 5 cultures each.

<sup>d</sup> The maximal yield for each set of salt proportions is shown in bold-face type.

that of group A3. The largest yield for both races was obtained with solution A6e, which is the best-balanced solution dealt with in this entire study. Two duplicate sets, of five cultures each, gave with this provisionally optimal solution an average male yield of 386 mg. and an average female yield of 414 mg. On the other hand, solution A3e, with much lower total salt

concentration but otherwise like solution A6e, gave yields of only 229 and 270 mg., for the male and female races respectively.

Markedly decreasing the total-salt concentration of solution B1d did not significantly alter the yields; this solution and solution B $\frac{1}{2}$ d gave practically the same yields.

In the solutions designated by T, with the three salts in nearly equal proportions and with dextrose concentration d (1.0 gram-mol. per liter), markedly larger yields of both races were obtained with the higher total-salt concentration numbered 4 (compare solutions T2d and T4d, table XI). The dry yields for both male and female races, as given in table XI, indicate that the salt proportions of group T are in much better physiological balance than are those of group C. The yields with solutions T2d and A2d are almost equal but the yields for solution T4d are much smaller than for solution A4d. While the higher total-salt concentrations of groups A and T gave markedly greater yields, it is apparent that the maximum yield should be sought with the salt proportions of group A.

(2) *The optimal solution for dry yields of the two races.* As has been mentioned, of all the solutions employed in this study, solution A6e gave unquestionably the highest yield for both races, which means that the process of accumulating non-aqueous materials in the fungus went on more rapidly with this solution as a medium than with any of the others. Solution A6e is better balanced for growth (as far as growth is measured by dry-yield production) than any other solution tested. It is probable that still more rapid production of dry yield might be obtained with still other solutions, perhaps with other temperatures, other oxygen conditions, etc., but, *so far as is now known*, this solution represents the physiological optimum for both of these two races of *Rhizopus*. It therefore becomes important to scrutinize the physical and chemical characteristics of solution A6e, which may be taken as criteria of the physiological requirements for most rapid growth, for the two races considered and for the non-solution conditions employed in this study.

As has been pointed out, the characteristics of any of the solutions tested may be considered as involving just three variables or sets of conditions: salt proportions, total-salt concentration, and dextrose concentration, these three characteristics being denoted by the three kinds of symbols used in designating the solutions. Solution A6e is characterized by the following three statements:—

Salt proportions A ( $\text{KH}_2\text{PO}_4$ , 6.0 :  $\text{NH}_4\text{NO}_3$ , 1.0 :  $\text{MgSO}_4$ , 1.1).

Total-salt concentration, 6 (corresponding to the calculated osmotic value of 14.5 atm.).

Dextrose concentration, e (1.0 gram-mol. per liter, osmotic value about 25 atm.).

(a) Considering these characteristics in order, the optimal solution of these studies has the molecular proportions of the two salts  $\text{NH}_4\text{NO}_3$  and  $\text{MgSO}_4$  about equal, with the partial concentration of  $\text{KH}_2\text{PO}_4$  about six times as great as that of either of the other two. All the ions excepting K, H and  $\text{PO}_4$  are thus supplied about equally, while the supply of K and  $\text{PO}_4$  is six times as great, and that of H is twelve times as great. But in the solutions with a high total salt concentration the values for the volume-molecular partial concentrations cannot be taken as an index of the number of ions that are available, as such, to the fungus, because none of the salts are completely ionized in such solutions. According to the data furnished by Jones and Tottingham, the approximate percentages of dissociation for the salts  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{NO}_3$  and  $\text{MgSO}_4$  in the concentrations employed in this solution are, respectively, 50, 88 and 48. Even after making allowance for the relatively low degree of ionization of  $\text{KH}_2\text{PO}_4$ , it appears that the ions K, H and  $\text{PO}_4$  are more numerous than any other ions in the solution. Furthermore, the undissociated salt molecules must be regarded as sources of supply for still more of these ions, which should appear in the solution as the fungus absorbed the ions originally present.

It is perhaps important to emphasize the relatively great need for K and  $\text{PO}_4$  to obtain this physiological balance. Of course, many sets of salt proportions not here tested might furnish still better conditions, if properly combined with other solution conditions, but it seems probable, from the nature of the four sets studied (A, B, C and T) and from the results obtained with them, that no very pronounced improvement over solution A6e may be obtained by other proportions of these three salts. It should be noted however that other sets of salts might be used to supply the same (or another) series of ions, and that out of the array of solution groups thus obtained (with various total-salt concentrations and various dextrose concentrations) some one or more might give greater yields, and so be better balanced, than the provisional optimum here considered. Six different sets of three salts are logically possible to furnish the seven ions here employed (neglecting Fe). These six sets of salts are shown below, the first set being the one used in this study. No tests have been made with any of the others.

$\text{KH}_2\text{PO}_4$	$\text{KH}_2\text{PO}_4$	$\text{KNO}_3$	$\text{KNO}_3$	$\text{K}_2\text{SO}_4$	$\text{K}_2\text{SO}_4$
$\text{NH}_4\text{NO}_3$	$\text{Mg}(\text{NO}_3)_2$	$\text{NH}_4\text{H}_2\text{PO}_4$	$\text{Mg}(\text{H}_2\text{PO}_4)_2$	$\text{Mg}(\text{NO}_3)_2$	$\text{Mg}(\text{H}_2\text{PO}_4)_2$
$\text{MgSO}_4$	$\text{NH}_4\text{H}_2\text{SO}_4$	$\text{MgSO}_4$	$(\text{NH}_4)_2\text{SO}_4$	$\text{NH}_4\text{H}_2\text{PO}_4$	$\text{NH}_4\text{NO}_3$

Furthermore, mono-hydrogen phosphate might be employed instead of the dihydrogen salt, thus giving six more possible salt series. A systematic comparison of these two phosphates might throw considerable light on the question of the influence of H-ion concentration or acidity upon the growth of these organisms. Also, it is not yet certain that all the ions employed in these cultures are necessary in the hypothetical actually optimal solution;

possibly solution A6e might be greatly improved by omitting one or more ions. It might be improved, also, by introducing other ions.

(b) The provisionally optimal solution (A6e) has a high total-salt concentration (osmotic value due to salts, about 14.5 atm.). According to the calculated osmotic values, this solution has next to the highest total-salt concentration employed in this study, solutions of the group C5 having the highest value (15.34 atm.). With the salts and sets of salt proportions here tested (and with the non-solution conditions and the dextrose concentrations that were involved in the present study), it appears improbable that a better solution than A6e might be found with any considerably *lower* total-salt concentration. But there is no evidence at hand as to whether, *ceteris paribus*, a still *higher* total salt concentration might, or might not, give a better solution for these fungus races, if such a solution could be prepared. There are, however, practical difficulties involved in maintaining the molecular salt proportions of solution A6e and increasing the total-salt concentration, for precipitation of some of the salts becomes marked when this is attempted.

(c) While some evidence is available (series II) regarding the optimal partial concentration of dextrose (with certain other solution conditions) it can not yet be said that a better solution than A6e might not be obtained with lower or higher dextrose concentrations than that here designated as e (1.0 gram-mol. per liter). With the non-dextrose conditions different in some way or other from those tested in this study, such a logical possibility must be considered. It should be noted that the salt combination A6 has not yet been tested with any dextrose concentration other than that designated by e. Also, dextrose is of course only one of a number of organic substances that might be employed. Numerous organic substances might be used alone or in various combinations, so that the possibilities of improving upon solution A6e in this direction are practically unlimited, as far as present knowledge extends. If the actually optimal solution is to be approached it will be necessary to test other soluble carbohydrates, etc., and also various amino acids, or substances that give these acids, such as peptone. Some tests employing glycerine instead of dextrose were made in this study, but the results do not warrant any suggestion that solution A6e might be improved by this substitution. These tests will be considered below.

Finally, it cannot be too strongly emphasized, in such a study as this, that *all* of the three characteristics of the nutrient medium must be considered together—the dextrose concentration, the salt proportions, and the total-salt concentration. Dextrose alone fails to support the growth of these *Rhizopus* races, and the three salts here used failed also when employed without dextrose (in all combinations of salt proportions and total-salt concentration tested). The physiological influence of any single condition or set of conditions is of course determined by the other effective conditions.

In this connection, it must be constantly remembered that the indications of the results presented in this paper are logically applicable only within the limits of the conditional complexes tested. Certain features, like oxygen supply, were not controlled at all in this work, and all conclusions arrived at are applicable only for the range of the oxygen condition that was employed. Other temperatures might also be tested if the actually optimal solution for these two fungus races was to be approached experimentally. Some special tests were made regarding the influence of temperature on the growth rate of these organisms and these will be considered below.

(3) *Comparison of the dry yields of the two races when grown under the same set of environmental conditions.* It appears from the results of series III (as in previous cases) that the two races must be regarded as about alike in their responses, by the criterion of dry yield, to all the solutions tested. However, the five solutions that produced the five largest yields in both races (table XI) actually showed a somewhat larger yield for the female race than for the male, in every case. The differences between the two races thus shown have been regarded as probably too small to be significant, and they have therefore been neglected.

### III. COMPARATIVE PHYSIOLOGICAL VALUES OF DEXTROSE AND GLYCERINE

To compare the physiological values of dextrose and glycerine for the two *Rhizopus* races here studied, the nine different salt combinations of series II (A1, 2, 3; B1, 2, 3; C1, 2, 3) were employed with 0.5 gram-mol. of glycerine per liter, in place of the dextrose used in series II. The usual sets of cultures were made with the male and female races being continued for a period of 10 days, with a temperature of 25°–26°C. The average dry yield per culture (from 5 cultures) is given for each of these nine solutions in table XII. For comparison, the corresponding data for the same concentration of dextrose with these same salt combinations are repeated from table VI.

Referring to table XII, glycerine is evidently not nearly as satisfactory in these combinations as is dextrose in the same partial concentration. A marked difference between the corresponding yields of the two races with glycerine is here shown, for the male race generally produced a significantly higher yield than did the female. This is the only culture series of this entire study in which the yields of the male and female races were distinctly and consistently different. That the two races are physiologically different in certain ways is of course clear from their conjugation and also from the difference shown in spore production (see p. 323). It is reasonable to suppose that a set of surroundings might be found in which the dry-yield production of the two races would be different, and it appears that one such set of surroundings was attained in this series of cultures with glycerine. So far as is known at present, these two races differ physiologically in three respects,

or by three criteria.<sup>21</sup> (1) They behave differently and complementarily in conjugating, with all the media that have been tested, where conjugation occurs at all. (2) They behave differently in the relative production of sporangia and vegetative hyphae with good or medium solutions such as those of series II. (3) They behave differently in the production of dry yield with the glycerine solutions here considered. This three-fold generalization constitutes a short step, but apparently a very definite one, toward an analysis of the physiological differences that are concomitant with the outstanding sexual difference between the two races.

Reverting to the matter of the relative physiological values of dextrose and glycerine, the latter substance has been reported by Hagem (1910) as of little or no physiological value in his cultures of various forms of *Mucor*.

TABLE XII

Data for comparison of the physiological values of glycerine and dextrose, when employed with the sets of salt proportions A, B and C and with total-salt concentrations 1, 2, and 3 (see series II, p. 28). Each yield is the average from a set of five similar cultures.

		AVERAGE DRY YIELD, MILLIGRAMS								
		Group A1	Group B1	Group C1	Group A2	Group B2	Group C2	Group A3	Group B3	Group C3
Dextrose, 0.5 gram- mol. per. liter	Male race	88	104	72	154	84	121	286	75	191
	Female race	80	99	66	154	72	116	271	76	165
Glycerine, 0.5 gram- mol. <sup>b</sup> per liter	Male race	42	31	42	30	64	36	129 <sup>a</sup>	29	34
	Female race	28	17	18	23	30	24	26 <sup>a</sup>	19	26

<sup>a</sup> These averages were obtained by averaging two sets of cultures with this solution. The yield for the male race is inconsistently high, but the average yields from the two sets of cultures for this race were actually 108 and 150 mg.

<sup>b</sup> The total osmotic value of each solution containing glycerine is of course equal to that of the corresponding solution with dextrose (see table V).

Dextrose seems to be well established as an excellent organic nutrient for many mucors, as well as for *Rhizopus* and numerous other fungi. Richards reported that *Penicillium* and *Aspergillus* gave about 50 per cent greater dry yields with dextrose as the organic nutrient than with glycerine, other conditions being alike, which is in general agreement with the results obtained with the two races of *Rhizopus* here studied. On the other hand, *Sporodinia* appears to be more vigorous in cultures with glycerine than in those with dextrose<sup>22</sup> as the source of carbon. Of course the relative nutritional values of glycerine and dextrose, or of any two or more organic substances, must depend upon the other environmental conditions (e.g., upon the kinds of

<sup>21</sup> Of course the male and female races of other strains of *Rhizopus nigricans* might not show these differences, and the races here dealt with may differ in many other respects not yet detected.

<sup>22</sup> Falk, R. Die Bedingungen und die Bedeutung der Zygotenbildung bei *Sporodinia Grandis*. Beiträge Biol. Pflanzen. 8: 213-303. 1902.

inorganic salts present, their molecular proportions and their total-salt concentration) as well as upon the partial concentrations of the organic substances themselves. Thus, it is still logically possible (though perhaps improbable) that a more vigorous growth of even the two *Rhizopus* races here studied might be obtained with glycerine than with dextrose, providing the other environmental features were properly adjusted.

#### IV. THE TEMPERATURE RELATIONS OF THE TWO RHIZOPUS RACES WHEN GROWN ON AGAR PLATES IN DARKNESS

A preliminary study was made of the temperature relations of the two *Rhizopus* races in darkness, the different culture groups being subjected to seven different, approximately maintained temperatures, in the temperature chambers described by Livingston and Fawcett.<sup>23</sup> Two like cultures of each race were grown in each temperature chamber, on nutrient agar plates (Petri dishes, 9 cm. in diameter and somewhat less than a centimeter deep). Inoculation was made by placing a single ripe sporangium in the center of the circular agar surface, all sporangia for each race being derived from a single stock culture and care being exercised to have them all as nearly alike as possible in each case.

The nutrient agar was prepared so that it contained salts in the molecular proportions and total concentration of group C3 (see table IV), with 0.02 volume-molecular dextrose. Its content of agar was 1.5 per cent.

At each time of observation measurements were made of two diameters of the fungus mat on the agar surface and these two measurements were averaged to give the mean diameter of the mat which is taken as the criterion of relative growth rate. Two series were carried out. The temperature used and the numerical data of mean diameters are set forth in table XIII.

The data of table XIII suggest that the optimal temperature for the growth of the male race lay between 25° and 28°C., while that for the female race appears to have been somewhat lower, at least for the longer periods. The data are too limited to permit of close approximations, however. The maximal temperature seems to have been about 31°C. and the minimum is clearly below 13°C.

These observations suggest a fourth criterion by which a physiological difference between the two races may possibly be demonstrated. The male race appears to have produced a measurable growth at a temperature slightly higher than the maximum for the female race. Also, the male race grew somewhat more rapidly than did the female at temperatures considerably below the optimum; e.g., 13° and 18°C.

---

<sup>23</sup> Livingston, B. E., and H. S. Fawcett. A battery of chambers with different automatically maintained temperatures. *Phytopathology* 10: 336-340. 1920.

Falek (1902) measured the growth rate of a form of *Rhizopus nigricans* (*Mucor stolonifer*) (presumably not either of the races dealt with in the present study) and recorded a fifty hour *radial* growth rate of 2 cm. with a temperature of 22°–24°C. This is equivalent to 4 cm. of *diameter* increase, which agrees very well with the rates shown in table XIII for the temperature 22°–23°C. and for the race here studied. This apparent agreement is not really so definite as it might seem and may well be considered as merely a coincidence, for it is not only true that Falek's fungus may have been different from either of the two races here studied, but his nutrient medium was entirely different from the one here used. His medium was a 2-per

TABLE XIII

*Growth rates of the two Rhizopus races on nutrient agar with different maintained temperatures, two series with somewhat different selected temperatures.*

FIRST SERIES				SECOND SERIES			
Maintained temperature	Length of period	Mean diameter of mycelial area		Maintained temperature	Length of period	Mean diameter of mycelial area	
		Male race	Femal race			Male race	Female race
deg. C.	hours	cm.	cm.	deg. C.	hours	cm.	cm.
18–19	48	2.5	2.4	13–13.5	48	1.0	0.9
	72	7.8	5.9		66	1.9	1.7
22–23	48	3.5	4.3	18–18.5	48	2.8	2.5
	72	9.0	9.0		66	5.9	3.5
26–27	48	5.3	5.3	24–25	48	4.6	4.5
	72	9.0	8.3		66	9.0	8.9
30.5–31	48	0.5	0	30	48	0.2	0.1
	72	0.7	0		66	0.2	0.2
36–37	48	0	0	0	0	0	0
	72	0	0		0	0	0

cent agar containing  $\text{MgSO}_4$ , KCl, dextrose, ammonium tartrate and peptone.

Hagem (1910) studied the relation of temperature to growth rate for a large number of species of *Mucor* derived from the soil, using nutrient agar in Petri dishes. His medium contained  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$ , dextrose and peptone. The maximum temperature for most of his forms lay between 25° and 35°C.; for *Rhizopus nigricans* (*Mucor stolonifer*) it was 32.5°C. As far as Hagem's evidence goes, the optimum for his strain of *Rhizopus nigricans* appears to have been between 20° and 25°C. Of course his organisms probably possessed more or less different internal conditions, or physiological characteristics, from those of the two races here studied, and his

nutrient medium was very different from those employed in the present study, so that anything more than very superficial comparisons are here out of the question.

Ames<sup>24</sup> reports that the optimal temperature for the growth of *Rhizopus nigricans* on agar containing an infusion of "string" beans is 36°C.

The various species of the genus *Rhizopus*, are not very clearly defined. And it seems that some of the discrepancies in the relation of temperature to the rate of growth of *R. nigricans*, as reported by various investigators, may be due to the fact that different strains or even different species have been employed. Besides the internal differences that would have to be considered if different strains were employed, there are also external conditions, as humidity and oxygen supply, that would necessarily differ considerably in studies conducted separately at different places,—unless, indeed, very special precautions were exercised. A classification of the species of *Rhizopus* based mainly on the temperature maximum, optimum and minimum, for growth and sporangia production, has been made by Hanzawa.<sup>25</sup>

After the study reported in this paper had been discontinued some observations were made on the relation of temperature to the rate of growth of several different strains of *Rhizopus*. The optimal temperature for the growth of the male and female races of *Rhizopus nigricans* on corn meal or potato-dextrose agar in Petri dishes (and with the other external conditions present during these later tests) was between 26° and 28°C. It was found that the maximum temperature for the germination of the spores was slightly higher for the male than for the female race. Also, at low temperatures (as 3°, 6° and 10°C.), male spores germinated more rapidly than did female spores.

With various strains of *Rhizopus* that are parasitic on strawberries and other fruits, it was found that there is considerable difference in the rates of growth of different strains at the same temperature and with all other external conditions similar. The optimal temperature for the growth of one strain was found to be about 36°C. while that for other strains was approximately 27° or 28°C. Likewise, there is a marked difference between these various strains or species in the rate of spore germination and of growth at low temperatures and in the maximal temperature for spore germination.

### GENERAL CONCLUSION

The main results obtained from this study of the two races of *Rhizopus nigricans* are presented in summary form in the Abstract appearing at the beginning of the present paper. They have a direct bearing upon the prob-

<sup>24</sup> Ames, Adeline. Temperature relations of some fungi causing storage rots. *Phytopathology* 5: 11-19. 1915.

<sup>25</sup> Hanzawa, J. Studien über einige *Rhizopus*-Arten. *Mykol. Centralbl.* 5: 230-247. 1914.—Different fungus forms appear to differ remarkably in their temperature requirements. For example, see: Fawcett, H. S., Univ. of California Publications, *in press*.

lem of the nutritional requirements of fungi in general and they give indications as to the characteristics of a liquid medium for optimal growth and spore production in the particular organisms studied. As has been emphasized, the experimentation of this study is not to be regarded as at all complete. Other influential conditions than these tested and other intensities of the conditions dealt with will require attention before any very definite or far-reaching conclusions may be reached.

## LITERATURE CITED

- AMES, ADELINE. The temperature relations of some fungi causing storage rots. *Phytopathology* 5: 11-19. 1915.
- BLAKESLEE, A. F. Sexual reproduction in the mucorineae. *Proc. Amer. Acad. Sci.* 40: 205-319. 1904.
- . Sexual reactions between hermaphroditic and dioecious mucors. *Biol. Bull.* 29: 87-103. 1915.
- . Sexuality in mucors. *Science* 51: 375-382, 403-409. 1920.
- FALCK, R. Die Bedingungen und die Bedeutung der Zygotenbildung bei *Sporodinia grandis*. *Beiträge Biol. Pflanzen* 8: 213-303. 1902.
- FREE, E. E. A relative score method of recording comparisons of plant condition and other unmeasured characters. *Plant World* 18: 249-256. 1915.
- HAGEM, O. Untersuchungen über Norwegische Mucorineen. II. *Vidensk. Selsk. Skr. (Math.-Naturv. Kl.)* 4: 1-152. 1910.
- HANZAWA, J. Studien über einige Rhizopus-Arten. *Mycol. Centralbl.* 5: 230-247. 1914.
- JONES, H. C. The electrical conductivity, dissociation and temperature coefficients of conductivity from zero to sixty-five degrees of aqueous solutions of a number of salts and organic acids. *Carnegie Inst. Washington Publ.* 170. 1912.
- LIVINGSTON, B. E., AND H. S. FAWCETT. A battery of chambers with different automatically maintained temperatures. *Phytopathology* 10: 336-340. 1920.
- MEDISCH, M. Beiträge zur Physiologie der *Hypocrea rufa*. *Jahrb. wiss. Bot.* 48: 591-631. 1910.
- MORSE, H. N. The osmotic pressure of aqueous solutions. *Carnegie Inst. Washington Publ.* 198. 1914.
- PFEFFER, W. The physiology of plants. Translated by A. J. Ewart. University Press: Oxford, 1900. Vol. 1, p. 432.
- PIETERS, A. J. The relation between vegetative vigor and reproduction in some Saprolegniaceae. *Amer. Jour. Bot.* 2: 529-576. 1915.
- RAULIN, J. Études chimiques sur la végétation. *Ann. Sci. Nat. Bot.* V, 11: 93-299. 1869.
- RICHARDS, H. M. Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. *Jahrb. wiss. Bot.* 30: 665-679. 1897.
- RICHTER, A. Zur Frage der chemischen Reizmittel. *Centralbl. Bakt.* II, 7: 417-430. 1901.
- SHIVE, J. W. A study of physiological balance in nutrient media. *Physiol. Res.* 1: 327-397. 1915.
- STEINBERG, R. A. A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. *Amer. Jour. Bot.* 6: 330-356, 357-372. 1919.
- TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* 1: 133-245. 1914.



# A PHYSIOLOGICAL STUDY OF THE CLIMATIC CONDITIONS OF MARYLAND, AS MEASURED BY PLANT GROWTH<sup>1</sup>

A SECOND CONTRIBUTION FROM DATA OBTAINED UNDER THE AUSPICES OF THE MARYLAND STATE WEATHER SERVICE, IN 1914

F. MERRILL HILDEBRANDT

## ABSTRACT<sup>2</sup>

The present paper presents the results obtained from a study of a series of observations on the climatic complexes for nine different stations in Maryland for the summer of 1914, as the effectiveness of each complex was automatically integrated by soy-bean plants grown for a period of 4 weeks from the seed, new seeds being planted every 2 weeks. Corresponding instrumental observations were also studied.

The field work was carried out by Dr. Forman T. McLean, under the joint auspices of the Maryland State Weather Service and the Laboratory of Plant physiology of the Johns Hopkins University. McLean<sup>3</sup> has presented an account of the plan and methods by which the observational data were obtained and he also made a thorough study of the growth data for soy-bean, for the stations at Oakland and Easton, his main results and conclusions having been set forth in his publications.

The study with which the present paper deals involved all the climatic and soy-bean data obtained by McLean; it thus included data for nine different localities well distributed throughout the state. The plants for all stations and for all periods were treated practically alike, excepting for the climatic conditions of the several localities. The same soil was used for all cultures. The values obtained from the plant measurements, made after 2 and after 4 weeks of growth, therefore constitute comparative and quantitative descriptions of the total influence exerted by all the climatic conditions upon the plants, and they exhibit the seasonal march of this climatic resultant in terms of the responses of the different sets of cultures. The plant measurements themselves, and the values derived from them, form perhaps the most important contribution in the present paper. The graphs of these values depict the seasonal march of each climatic complex, not after the manner of the instrumental readings usually employed in the study of climate, but as measures of the effectiveness of the climatic complex to favor or retard the growth of the standard plant, the latter being employed as a "living instrument" or indicator of climatic effectiveness.<sup>4</sup>

The plant measurements employed are: (1) stem height, (2) leaf area, (3) leaf-product (length multiplied by width), (4) dry weight. The climatic values used are: (1) air temperature, (2) the evaporating power of the air, (3) sunlight intensity and duration. Values to represent the temperature efficiency for growth are derived from the temperature records by means of the Livingston physiological temperature indices

<sup>1</sup> Botanical contribution from the Johns Hopkins Hospital University, no. 60. This work was practically completed in 1917, but war conditions delayed its publication.

<sup>2</sup> This abstract was preprinted, without change, from these types and was issued as *Physiological Researches Preliminary Abstracts*, vol. 2, no. 8, May, 1921.

<sup>3</sup> McLean, Forman T. A preliminary study of climatic conditions in Maryland, as related to plant growth *Physiol. Res.* 2: 129-208. 1917. A brief account had appeared earlier:—*Idem*. Relation of climate to plant growth in Maryland. *Monthly Weather Rev.* 43: 65-72. 1915.

<sup>4</sup> Livingston, B. E., and McLean, F. T. A living climatological instrument. *Science* 43: 362. 1916

All of the measurements, both plant and climatic, are expressed relatively, so that they may be compared for different stations and for different periods.

Considering the soy-bean plant (as here employed) as a standard plant or indicator for the measurement of climatic efficiency to produce plant growth, it appears that, for the season of 1914, the climatic complexes for some culture periods were more efficient in this sense than might be expected from an attempt to interpret the corresponding climatic data, while the complexes for other periods were actually less efficient to produce soy-bean growth than might be surmised from the corresponding climatic values. No successful method has yet been brought forward by which the value of a climatic complex, to produce growth in any plant form, may be deduced from instrumental data, and the plant measurements of this study furnish a means by which climatic efficiency as a whole may be directly compared for different periods and for different stations. The seasonal means derived from the plant data give relatively low efficiency values for the climatic complexes of the three western stations (Oakland, Chewsville and Monrovia), high values for the complexes of Baltimore and Darlington, and intermediate values for the complexes of the remaining stations (College, Coleman, Easton, and Princess Anne).

If indices for total seasonal climatic efficiency are derived by multiplying the seasonal average growth rate per day by the normal length (days) of the growing season for the station in question, these indices have the following values for the several stations: Oakland, 9009; Chewsville, 12480; College, 16867; Easton, 17688; Princess Anne, 19005; Coleman, 21115; Darlington, 23688; Baltimore, 25422. In considering these relative climatic indices it should be emphasized that the data of this study do not involve precipitation as an influential climatic feature; the culture plants were automatically irrigated so that they never suffered from lack of soil moisture.

A study was made of the interrelations of the different kinds of plant measurements, dealing therefore with some aspects of growth correlation in soy-bean. It appears that the rate of stem elongation was greater than the rate of leaf expansion when both were relatively small, while the former rate was the smaller of the two when both were relatively large. The rate of production of dry weight appears to have been nearly proportional to the rate of increase of leaf surface; the relative values of these two growth criteria are generally about equal numerically.

The statements just made apply to the data for plants quite openly exposed, but some observations on cultures somewhat protected above by glass were available, and also a set of observations on cultures in forest at Baltimore. These all indicate that the height rate was relatively greater than the rate of leaf expansion for these more or less shaded conditions, while the rate of dry-weight production was smaller than the corresponding rate of leaf expansion. The outcome of this part of the study may throw some light on the general problem as to what sort of plant measurements may be best suited to quantitative comparisons of the efficiencies of different climatic complexes. An interesting, and probably valuable result of this study is that the calculated leaf-product (length times breadth, which can be obtained without injury to the plants, if that is desirable) is generally proportional to the leaf area; of course for these soy-bean plants.

The climatic data themselves showed a pronounced general agreement between the graph for sunlight and the corresponding one for evaporation (standardized white cylindrical porous-cup atmometer), this being probably due to the relatively great importance of solar radiation in determining the evaporation rate. The climatic values indicate a general seasonal march, which is very evident for temperature, less so for sunlight and rather obscure for evaporation. For details regarding the climatic values, as well as for the plant values, reference must be made to the tables and graphs and to the text of the paper.

## CONTENTS

Introduction.....	344
The observational data and the averages derived from them	
The plant measurements.....	349
The climatic measurements.....	352
Temperature.....	353
Light.....	357
Evaporation.....	359
Results and discussion	
Introductory.....	360
Results from stations in the open	
The 2-week values	
The 2-week plant data for stations in the open	
Correlations between the 2-week plant graphs.....	371
Trends of the 2-week plant values and their seasonal ranges for the several stations.....	376
The 2-week climatic data for stations in the open.....	377
The 2-week temperature data.....	378
Chewsville and Monrovia.....	378
Baltimore, Darlington and Coleman.....	379
Easton and Princess Anne.....	379
College.....	379
Oakland.....	379
The generalized graph.....	380
Light and the evaporating power of the air, 2-week data.....	380
Variability of temperature and evaporation values.....	382
Correlation of the 2-week plant and climatic values.....	383
The 4-week values.....	385
The 4-week plant data for stations in the open.....	386
The 4-week climatic data for stations in the open.....	390
Results for the three covered stations	
Introductory.....	391
The plant data, covered stations.....	391
The Oakland covered station.....	393
The Baltimore covered station.....	394
The Easton covered station.....	394
The climatic conditions, covered stations.....	394
Results for the Baltimore forest station.....	395
The plant data as measures of the climatic efficiency for growth of the standard plants	
Introductory.....	396
Seasonal averages of mean daily intensity values for the several stations....	398
Total seasonal efficiencies for the several stations.....	403
General conclusion.....	405

## INTRODUCTION

During the summer of 1914 an elaborate investigation was undertaken by the Maryland State Weather Service in cooperation with the Laboratory of Plant Physiology of the Johns Hopkins University, with the object of ascertaining some of the relations between climatic conditions and the growth of certain plants exposed at different stations in Maryland. Detailed information as to the growth of the plants used is of course necessary in such a study, as is also corresponding knowledge of those environmental conditions that are considered as climatic. The plant records were secured in this case by growing cultures of certain plants under the environmental conditions to be studied, and noting the amount of growth accomplished during definite periods of time. In order that a corresponding series of measurements of some of the environmental conditions might be available for comparison with these growth measurements, the cultures were located at certain of the regular observation stations of the U. S. Weather Bureau, at nine different places in the state. The general plan of the study and a detailed consideration of the methods used has already been published by McLean,<sup>5</sup> who did all of the field work personally. The original data dealt with in the present paper were secured from McLeans' records, and it will be necessary to give here only so much description of the ways in which these measurements were obtained as is needed to render their use in the present publication intelligible. The following description is taken mainly from McLean's paper, which also deals with the growth of soy-bean plants, but for only two of the stations, Easton and Oakland. The present paper gives the main results for soy-bean plants for all of the nine stations, together with some attempts at interpretation.

This study has been carried out partly through financial aid furnished by the Maryland State Weather Service. It was suggested by Prof. B. E. Livingston and carried out under his direction. The writer wishes to express his indebtedness to Prof. Livingston for much assistance in carrying out the study and for helpful criticism in the preparation of manuscript. The writer also wishes to thank Dr. H. E. Pulling for valuable suggestions made during the course of the study.

The stations employed were Oakland, Chewsville, Monrovia, College Park, Baltimore, Darlington, Coleman, Easton, and Princess Anne. One station, Oakland, is in the Allegheny plateau. Four stations are in the Piedmont plateau; one of these (Chewsville) being in the Hagerstown valley, two (Darlington and Monrovia) in the hilly country north and west of Baltimore, and one (Baltimore) at the lower edge of the plateau near Chesapeake bay. Four stations, College Park, Coleman, Easton and Princess Anne, are in the coastal

<sup>5</sup> McLean, F. T. A Preliminary study of climatic conditions in Maryland, as related to plant growth. *Physiol. Res.* 2: 129-208. 1917.

plain. Coleman, Easton, and Princess Anne, are east of Chesapeake bay, while College Park is west of it and much farther inland, near the line of demarcation between the coastal plain and the Piedmont plateau. All of the stations except Oakland are at comparatively low elevations—less than 310 meters (1000 feet) above sea-level. Oakland has an elevation of 775 meters (2500 feet). The geographical distribution (see fig. 1) of these stations is such as to insure considerable differences in climatic conditions.

At each of the nine places referred to, a series of cultures was grown in the open with no covering other than a screen of large-meshed wire netting. These have been termed the exposed stations. Also, at Oakland, Baltimore, and Easton series of cultures were grown under glazed cold-frame sash supported horizontally 1 meter (3.3 feet) above the surface of the soil. These have been termed the covered stations. The plants were freely exposed at the sides, the sash being supported merely by corner posts. They were located within a very few meters of the enclosures containing the plants of the exposed stations and were subjected to the same climatic conditions as the exposed plants, except in so far as these conditions were modified by the glass covers. Furthermore, a series of cultures was exposed in the woods near the Laboratory of Plant Physiology of the Johns Hopkins University at Baltimore. This has been termed the Baltimore forest station. As in the case of the exposed stations, these forest cultures were covered by a protective screen of wire netting. They were, of course, subjected to a complex of climatic conditions quite different from those of the exposed and covered stations at Baltimore. The forest station was distant about 150 meters (490 feet) from the exposed and covered stations, at Baltimore. There are thus plant data available from 13 series of cultures in all, each series having been exposed to a different set of environmental conditions throughout the season.

The cultures were so planned that the plants might be considered as standard plants for the comparative measurement of climatic conditions in accordance with a suggestion made by Livingston and McLean.<sup>6</sup> Since the problem of expressing plant growth in terms of the instrumental measurements of the climatic conditions that control it is rendered exceedingly complex by the number of these conditions and by their continual variation, as well as by the changing internal conditions of the plant itself, a detailed analysis of the control of plant growth is very difficult. Livingston and McLean suggest that the rate of growth of any plant is itself an expression of the summation of all the effects of the external conditions acting during the growing period, and that a standard plant may be employed as an automatically weighting, integrating, and recording instrument for the comparative measurement of environmental conditions as these are effective in growth control. Thus several environments may be measured and compared in terms of their sev-

---

<sup>6</sup> Livingston, B. E., and McLean, F. T. A living climatological instrument. *Science* 43: 362. 1916.



eral capacities for producing growth in the standard plant. This method of measuring the environmental complex in terms of plant growth can be applied, of course, only when it may be assumed that all the standard plants are alike at the beginnings of the several periods of exposure. In this study the requirement just stated was fulfilled by employing the seed as the starting point for the plants of the various cultures. It was apparent that if the cultures were always started from the seed, the plants might be considered as more nearly alike at the beginning of the several culture periods than would have been the case if an attempt had been made to obtain like plants in any other phase of their development.

Growth rates were measured and compared in terms of size and weight of the plants. Each culture consisted of six plants grown for a period of four weeks from the seed. Cultures were started approximately every two weeks during the growing season, at each of the stations employed, and growth measurements were made after about two weeks and again after about a month. The plants were harvested at the end of the longer period.

As in other problems in which a number of conditions enter into the control of a process, the relations between conditions and process rate are more easily detected the smaller is the number of conditions involved, and conditions may be temporarily left out of consideration if they are the same in several experiments. Just as the internal conditions of the standard plant at the start of the experiment are left out of the argument by the simple device of having them all alike at the beginning of the exposure period (the instrument being thus set at the zero of its scale, in the words of Livingston and McLean), so selected conditions of the surroundings may be left out of consideration by having them alike throughout all of the periods. According to this principle, most of the environmental conditions that acted on the plants below the soil surface were kept nearly the same for all times and at all stations. Assuming that this artificial control of the subterranean environmental conditions kept them practically constant, the differences observed in the growth rates of the standard plants were taken to be related almost entirely to the aerial conditions of the surroundings. These are the ones referred to by McLean as *climatic*, and this term will be used with the same meaning in the present paper. To accomplish this control of the subterranean conditions, the soil was always the same at the beginning of all cultures and its moisture content was generally kept approximately the same throughout all culture periods, by means of the Livingston auto-irrigator. This arrangement and its operation have been described by McLean and will also receive some attention below.

While several different plant species were employed throughout the experimental work, the present paper deals only with the data obtained from soybean. A variety of this plant called "Peking," was used. The seed was of pure strain obtained from the 1913 crop of the Maryland Agricultural Experi-

ment Station. All the seeds were first treated with carbon bisulphide vapor for one week, to destroy insects, after which they were placed in paraffined paper cylinders with tight-fitting covers and stored until ready for use.

A rather light soil, of the type classified as Norfolk Sand by Bonsteel,<sup>7</sup> obtained from an untilled field near College Park, Maryland, was used in all of the plant cultures. This soil was chosen for three reasons: first, its water-holding power is comparatively low, so there was little danger of long-continued excessive water-logging after a rain; second, it was not a very fertile soil and yet was capable of giving good growth under favorable climatic conditions; and finally, it was conveniently obtained. The method of obtaining it was to remove the top soil to a depth of 15 cm. from a small area of the field. This top soil was then thoroughly mixed, sifted, and placed in cloth sacks for shipment to the various stations where it was stored in air-dry condition in covered, water-tight, galvanized iron cans until needed for use in the cultures. The soil containers for the cultures were ordinary "6 inch" porous clay flower-pots, in form like the frustrum of a cone, being smaller at the bottom, and of a cubic capacity of approximately 1980 cc.

In order to secure uniform soil conditions in the various cultures, it was necessary, not only that the soil should be of the same character in all of them, but also that it should be brought into the same physical condition for the beginning of all cultures. Furthermore, it was desirable that this physical condition be such that it might be retained with as little change as possible during the growth periods of the plant. To put the soil into a state of aggregation to be least altered by varying weather conditions (especially heavy rains which pack it more or less) it was saturated with water immediately after being put into pots. This was accomplished by plunging the filled pots into a bucket of water and allowing them to remain submerged until air bubbles ceased to rise. The pots were then drained and a two-week interval was allowed to intervene before the seeds were planted.

The soil moisture in the cultures was maintained always *above* a certain minimum by means of auto-irrigators.<sup>8</sup> This device, as here used, consisted of two cylindrical porous clay cups (of the regular form supplied by the "Plant World") connected with each other and with a water reservoir by glass tubes each having the form of an inverted J. The cups were placed vertically in the pot, their rubber-stoppered tops level with the soil surface, and were so arranged as to supply water to the soil against a pressure of 35 cm., or somewhat more, of a water column. The moisture content of the soil was thus maintained so that it was never less than about 10 or 11 per cent on the basis

<sup>7</sup> Bonsteel, J. A. The Soils of Prince George's County [Maryland]. Maryland Geological Survey: Baltimore, 1911.

<sup>8</sup> Livingston, B. E. A method for controlling plant moisture. Plant World 11: 39-40. 1908. Livingston, B. E. Auto-irrigation of pots of soil for experimental cultures. Carnegie Inst. Washington Year Book 14: 76. 1916.

of dry weight. With this water content this particular soil was rather too wet than too dry for the best growth of the plants.

After preparing the pots and arranging the watering devices the pots were allowed to remain fallow for about two weeks before planting, as has been mentioned. Thus the soil was fully drained after the preliminary saturation and had settled into a condition somewhat approaching that of structure equilibrium, before the seeds were planted. The seeds were placed 2.5 cm. deep, six seeds in each pot. Care was taken to space them uniformly from each other, from the auto-irrigator cups and from the sides of the pots, so that all should have, as nearly as possible under the general conditions of the experiments, the same soil moisture conditions. When the plants were removed from a pot (about six weeks after that pot had been filled) the soil was discarded and fresh soil from the stored supply was used in refilling for the next following culture.

## THE OBSERVATIONAL DATA AND THE AVERAGES DERIVED FROM THEM

### THE PLANT MEASUREMENTS

The first plant measurements were made after approximately two weeks of growth from the seed. At that time the length of each leaflet, from the tip to the junction of blade and petiole, was determined, as was also the greatest width of each leaflet, measured at right angles to the long axis. The height of each plant was also measured, from the soil surface to the base of the terminal bud. At the end of approximately four weeks of growth the height measurement was repeated, after which the plants were cut off at the soil surface and photographic prints were prepared of the fresh leaves. By means of these leaf-prints the leaf area (one side) was afterwards determined planimetrically, for each leaf and for each plant. Finally, the dry weight of tops for each culture was determined. All linear measurements were made to the nearest millimeter, areal measurement to the nearest 0.1 sq. cm., and weight measurements to the nearest 0.01 g.

For convenience, these five kinds of observational data on growth rates are summarized below.

*After about 2 weeks of growth:—*

- (1) Stem, height, millimeters.
- (2) Length and breadth of all leaflets, millimeters.

*After about 4 weeks of growth:—*

- (1) Stem height, millimeters.
- (2) Leaf area (one side), square millimeters.
- (3) Dry weight (of tops), milligrams.

Of course the growth data obtained by actual observation require averaging in some way so as to represent the various periods throughout the season. The ordinary method of averaging was applied in all cases, but the observational data for leaflet dimensions required special preliminary treatment. The two dimensions derived from the measurements were multiplied together for each leaflet, giving the leaflet-product and these products were summed to give the total leaf-product of each plant. As McLean pointed out, the mean daily rate of increase in total leaf-product for a period of 4 weeks is very nearly proportional to the corresponding rate of increase in actual leaf area, and it seems safe to suppose, as McLean did, that the 2-week leaf-product values may be regarded as indices of increase in the area of the leaves. These product values are therefore used, for the 2-week periods of this study, as indicators of the rates of leaf expansion. Leaf areas were of course not determined for the 2-week periods, although they are available for the 4-week periods.

There were six plants started in each culture, but in many cases the number of plants from which records were actually taken was less than six (it was never less than three and was usually four or five in such cases), on account of noticeable injury due to other conditions than the ones here studied, such as insect attack, etc. All plant data have therefore been reduced to averages per plant. Also, in many cases the length of the period differed slightly from 14 days for the 2-week periods, and from 28 days for the 4-week periods, and the averages per plant have consequently been expressed as *mean daily values* for the respective periods. This method renders the plant measurements for the different periods more strictly comparable. It should be noted, however, that the growing periods were 14 and 28 days long in the majority of cases, and that variations in the length of the culture period were slight. Considering the 2-week and 4-week plant values as measures of the results of plant processes acting through these periods, the mean daily values represent mean daily increments or process rates, and they will be termed "daily increments," for their respective periods, in the discussion that follows. Thus, for a plant 10 cm. high at the end of a 13-day period, 10/13 (or 0.77) cm. is regarded as the mean daily increment of increase in height, for that period. Letting the word *growth* represent the particular process to which the given kind of measurement refers (as increase in height, increase in leaf area, etc.), these may be spoken as *growth increments*.

The mean daily growth increments, and also the mean daily climatic values, for the respective periods have been expressed in terms of the corresponding average of *all the periods considered*, for all exposed stations. This procedure renders all the values directly comparable. To obtain this unit for any kind of value, all of the corresponding values (as all 2-week daily mean increments in height, for example, for all exposed stations) were summed and the sum was divided by the number of values summed. Then each indi-

vidual value was divided by the unit thus obtained. The data were expressed as these ratio values, which will be termed *relative values* in the following discussion. To avoid decimals, these relative values have all been multiplied by 100, and are thus given in the tables and in the text of this paper. The absolute magnitude of the unit used for expressing each kind of value is of course not important; it is essential only that all comparative values be expressed in terms of the same unit. The unit here employed represents in every case simply the average of all similar quantities that are used in the present study. If another station had been employed, or if the season had been longer or shorter at any station, the values of these comparative units would have been different. The magnitudes of these units thus depend to some extent upon the climatic conditions encountered at the various stations in the summer of 1914, to some extent upon the number and location of the stations, to some extent upon the nature of the soil used in this investigation, and to some extent upon the physiological nature of the soy-bean plant. The actual values of the various growth units are given below.

*For first 2 weeks of growth from seed*

Average daily increment of stem height per plant, 3.56 mm.

Average daily increment of leaf-product per plant, 112.0 sq. mm.

*For first 4 weeks of growth from seed*

Average daily increment of stem height per plant, 3.20 mm.

Average daily increment of leaf area per plant, 122.0 sq. mm.

Average daily increment of dry weight (of tops) per plant, 6.29 g.

The use of the relative values described above simplifies the plotting of the graphs upon which interpretation of such a study as this so largely depends. It also renders possible a direct comparison between the values for any two cultures irrespective of their dates or stations. Furthermore, it is possible to tell from the magnitude of the relative value for any culture the extent to which the plant, or climatic, measurement under consideration departs from the mean of that measurement for all the cultures of the study.

To obtain the original or absolute plant, or climatic, value from a given relative value, it is necessary only to reverse the arithmetical procedure by which the relative value was derived. For example, suppose it is desired to get the actual mean daily rate of increase in leaf-area per plant for the four-week period ending September 2, and for the station at Coleman. The relative value given in the table is 108. The first operation is to divide by 100, which gives 1.08 as the true relative value. The average daily increment in leaf area per plant, for the period and station in question, was therefore 1.08 times the value of the common unit employed for comparing the rates of increase in leaf area. Multiplying this unit value (122 sq. mm., as given above), by 1.08 gives 132.0 sq. mm. as the actual mean daily increment required. To obtain the average total leaf area per plant at the end of the period in question, we multiply 132.0 sq. mm. by the number of days in the period (28 in this

case) and get 3698 sq. mm. Since there were 5 plants measured in this culture, the total leaf area for the entire culture at the end of the period is obtained by multiplying 3698 sq. mm. by 5, which gives 18490 sq. mm. or 184.9 sq. cm., which is the actual areal value determined from the prints of these leaves. All of the original absolute values may be obtained from the relative ones in a similar manner. It is of course evident from the above description of the manner in which the relative values have been derived that they are proportional to the corresponding absolute values. In all subsequent discussion, when plant (and also climatic) values are referred to, it will be understood that these are the relative values rather than the absolute ones.

As in the case of the observational data, the five kinds of derived growth values used in this study are listed below, for convenience.

*After about 2 weeks of growth from seed*

- (1) Relative mean daily increment of stem height per plant.
- (2) Relative mean daily increment of total leaf-product per plant.

*After about 4 weeks of growth from seed*

- (1) Relative mean daily increment of stem height per plant.
- (2) Relative mean daily increment of total leaf area per plant.
- (3) Relative mean daily increment of dry weight (tops) per plant.

### THE CLIMATIC MEASUREMENTS

The weather observations taken by the coöperative observers at the several stations here employed consisted of daily readings of maximum and minimum thermometers, daily ocular observations of cloudiness, daily measurements of rainfall, and general notes as to storms, wind, etc. The records of the Baltimore office of the Weather Bureau were used for the Baltimore stations. In addition to these records of the weather observers, evaporation was measured by means of Livingston standardized cylindrical porous cups with non-rain absorbing mountings.<sup>9</sup> Of the five sets of climatic observations mentioned above, only three will be considered in this paper; namely those of temperature, light, and evaporation. As was pointed out by McLean, rainfall showed little or no relation to the growth of these plants, as was indeed to be expected, since the soil moisture of the cultures was always kept sufficiently high (by the auto-irrigators) for the needs of the plants. Also, the miscellaneous climatological observations reported by the weather observers will not be considered in this paper, since none of them have been found to bear any discoverable relation to the growth rates of these plants. The

<sup>9</sup> Livingston, B. E. A rain-correcting atmometer for ecological instrumentation. *Plant World* **13**: 79-82. 1910.—Livingston, B. E., and Shive, J. W. The non-absorbing atmometer. *Carnegie Inst. Washington Year Book* **13**: 93-94. 1915.—Shive, J. W. An improved non-absorbing porous cup atmometer. *Plant World* **18**: 7-10. 1915.—Johnston, E. S. A simple non-absorbing atmometer mounting. *Plant World* **21**: 257-260. 1918.—Livingston, B. E., and Thone, Frank. A simplified non-absorbing mounting for porous porcelain atmometers. *Science* **52**: 85-87. 1920.

observational data were obtained daily throughout the entire season. For convenience, these three kinds of observational data on climatic conditions are listed below.

(1) Temperature.

Daily maximum and minimum air temperature (shade), degrees, Fahrenheit.

(2) Light.

Daily light condition, whether clear, partly cloudy, or cloudy.

(3) Evaporation.

Daily evaporation from standardized cylindrical porous-cup atmometer, cubic centimeters.

The climatic data, like the plant data, require special treatment before they can be used in such a study as this. How the mean values for the various periods were secured from the observational data will be described, for each of the three kinds of climatic measurements, in the following sections.

#### TEMPERATURE

It is clear that the readings of a thermometer do not express the effectiveness of various degrees of temperature to accelerate or retard plant growth, and it therefore becomes desirable to replace the actual thermometer readings by a series of weighted values, more or less directly proportional to the temperature effect upon the growth of plants. Owing to lack of information of a quantitative nature as to the relation between plant growth and environmental temperature, this can be accomplished only in a tentative and approximate way at the present time.

The observational data for temperature were all obtained from maximum and minimum thermometers read daily at sunset, these data being taken from the published monthly reports of the U. S. Weather Bureau.<sup>10</sup> The mean temperature for each day was determined by averaging the maximum and minimum for that day. McLean has discussed some of the ways in which daily maximum and minimum temperature data may be treated in order to obtain weighted values that may tentatively represent temperature effects upon plant growth rates. He emphasizes the fact that temperature values, as shown by a thermometer, do not show a linear proportionality to plant growth. If thermometer readings might be taken as expressing, even in an approximate way, the effectiveness of temperature to produce plant growth, such a relation could only be true up to the optimum temperature, since beyond this point *increased* temperature results in *decreased* growth. It would therefore be desirable to replace each thermometer reading by an *index* representing the effectiveness of that particular temperature for plant

<sup>10</sup> Fassig, O. L. Climatological data, Maryland and Delaware Section. May to November, inclusive. U. S. Weather Bureau. 1914.

growth. Three ways of doing this, all of which have been considered by McLean, may receive brief mention here.

(1) One way of expressing temperature, which has been used in ecological studies, has been called the *remainder-summation* method. This is based on the supposition that the growth activities of most plants stop when the temperature falls below 40° F.<sup>11</sup> Above this temperature, growth increases with increased temperature, to an optimum. For convenience, the growth rate for 40° F. may be considered as unity: then it should be 2 for 41°, 5 for 44°, 20 for 59°, etc. If we subtract 39° from any given temperature, the remainder will represent, according to this method, the *efficiency* of the given temperature for producing growth. A total efficiency value for any period of time, such as the 4-week growth periods of these studies, may be obtained by subtracting 39° from each daily mean temperature and summing the remainders for the period, thus obtaining the remainder summation.

(2) Another method of weighting temperature values for the purpose before us, and one that apparently has a somewhat more rational basis, was suggested by Livingston and Livingston.<sup>12</sup> They proposed a series of temperature-efficiency indices based on the van't Hoff-Arrhenius law, which states that the velocity of many chemical reactions approximately doubles with a rise in the temperature of 10°C. (18° F.). If it is assumed that the growth rate for plants follows this law above 40° F., at which temperature the rate is taken to be unity, a series of values representing temperature efficiencies for higher temperatures may be derived. When this scheme is used, the efficiency value for any temperature is represented by the value of the index that corresponds to the temperature value itself. Assuming the growth rate to be unity for a temperature of 40° F., it should be 1.21 for a temperature of 45°, 2.0 for 58°, etc. These indices are called by Livingston and Livingston "exponential indices," and they have published a table of these values.

Since most of the temperatures with which we have to deal are below the optimum for plant growth, since temperature and the growth rate are related in an approximately linear manner between 40° F. and the optimum (about 90° F.), and since both the exponential and remainder series of index values increase in a nearly linear way throughout this range, both of the methods just considered give temperature efficiency numbers that are more or less approximately proportional to plant growth as it is influenced by temperatures between these limits. It is obvious, however, that neither of these methods can properly express efficiencies for temperatures above the optimum,

<sup>11</sup> The temperature data of this paper are all expressed in terms of temperature degrees on the Fahrenheit scale, simply because the observational data had this old-fashioned form. By retaining the Fahrenheit values much labor has been avoided, but it is not to be understood that the writer is really as conservative as this feature of the paper might seem to suggest.

<sup>12</sup> Livingston, B. E., and Grace J. Livingston. Temperature coefficients in plant geography and climatology. Bot. Gaz. 55: 349-375. 1913.

since they give numbers which continue to increase with increasing temperature, while growth increases with increasing temperature up to the optimum and then decreases with higher temperature. Also, both these methods give results that are approximately proportional to each other for ordinary summer temperatures. This fact has been noted by Livingston and Livingston and again by Stevens,<sup>13</sup> and it is also obvious from the climatic data given by McLean. But it must be remembered that these methods cannot be satisfactory excepting when the temperatures dealt with lie mainly between 40° and 90° F.

When the exponential indices are employed each daily mean temperature for any period is replaced by its index and the series of indices thus obtained is summed for the period, giving the exponential summation.

(3) The third method of expressing temperature values as they affect plant growth has been more recently suggested by Livingston.<sup>14</sup> It is based on the results of Lehenbauer's experiments with maize seedlings. From Lehenbauer's data, Livingston derived a series of coefficients giving the efficiencies of various temperatures in terms of the growth of this plant. He has called these "physiological temperature indices." The growth rates upon which the index values were based are those shown by Lehenbauer's seedlings when exposed for 12 hours to various maintained temperatures, the other conditions of the experiment being approximately the same for all tests. Livingston suggests that the coefficients thus derived from the growth of maize under controlled conditions, with different maintained temperatures, may possibly express some approach toward a general relation between plant growth and temperature and may thus be applicable to plants growing under other conditions. The graph of these physiological indices exhibits the same direction of slope between a low temperature and the optimum as do the graphs of temperature efficiencies derived by the other two methods, but for this portion of the temperature range the slope of the graph of physiological indices is generally steeper than that of the graph of remainder indices, the latter graph itself having a much steeper slope than that of the exponential indices. This is shown by Livingston and also by Stevens, in the papers cited above. Since they are derived from the actual growth rates of a plant, the physiological temperature indices appear to have a more rational basis than either the remainder or the exponential indices. For this reason, and for others that will appear below, physiological indices have been used in this study for expressing the temperature values as they are to be compared with the plant growth-rates.

<sup>13</sup> Stevens, Neil E. Influence of temperature on the growth of *Endothia parasitica*. Amer. Jour. Bot. 4: 112-118. 1917.—*Idem*. Influence of certain climatic factors on the development of *Endothia parasitica*. *Ibid.* 4: 1-33. 1917.

<sup>14</sup> Livingston, B. E. Physiological temperature indices for the study of plant growth in relation to climatic conditions. *Physiol. Res.* 1: 399-420. 1916.—Lehenbauer, P. A. Growth of maize seedlings in relation to temperature. *Physiol. Res.* 1: 247-288. 1914.

In using the physiological temperature indices for the purposes of this study, the procedure has been as follows. Each daily mean temperature for any period has been replaced by its corresponding index (taken from Livingston's table,—1916) and then all the daily index values have been summed to give the physiological summation for the period. This summation value is finally divided by the number of days in the given period.

Two other series of temperature values are presented in the tables of this paper, but neither has been found to be as satisfactory for expressing this climatic condition as are the physiological-summation indices. These are (a) the average daily mean temperature for each culture period (in degrees, Fahrenheit) and (b) the remainder-summation index for each period.

As has been stated, the physiological-summation indices for all periods have been represented as daily means, and these have been stated always as relative values, in terms of the general average for all periods and stations. The average value used as unity in expressing the relative index values for temperature is 56.39.

The following considerations may be added to show the reason for using the physiological-summation indices in this study, secured as above described, rather than the remainder-summation or exponential-summation indices. It will be necessary to anticipate somewhat the discussion that is to follow this section. The three climatic conditions (temperature, evaporation and light) each show a definite seasonal march for each of the places employed in this study. The temperature rises from low values in the spring to a mid-summer maximum, which is followed by a subsequent fall to low autumnal values. On the other hand, the values representing light and evaporation both decrease, in general, throughout the season. If, now, a generalized curve representing the growth of the plants be drawn, employing average values to represent all the stations together, and plotting them as ordinates with the dates of the middles of the periods as abscissas, such a growth curve follows the seasonal march of *temperature* and shows only secondary variations as related to the other two climatic indices. The growth of the plants is thus apparently determined mainly by temperature. Obviously, also, the seasonal march of the temperature values must show the same general form of curve no matter what scheme is used in expressing temperature efficiency. In view of these facts, and in consideration of the general comparative purpose of the present study, a method should be used, for expressing temperature efficiency, that gives a seasonal march of the efficiency values in accord with the corresponding march of generalized plant growth. Of the three methods mentioned, the physiological-summation index fulfills this requirement best, and this has accordingly been selected for use throughout the entire study, as has been said.

An examination of the plant and climatic graphs (to be considered later) shows that the plant values for most of the stations rise above the temperature

efficiency values in the middle of the season, and fall below them, at its end. This is probably due in part to the effect of light and evaporation but it may also be related to an inadequacy of the temperature efficiency values to represent the actual effect of temperature on the growth of these plants. It appears to be at least suggested that the actual temperature efficiency values for these soy-bean plants increase more rapidly with increase in the temperature itself, for the range here encountered (between 40° and 85° F.), than to the physiological index values derived from Lehenbauer's study of maize seedlings. This whole question deserves much more experimental study. It is a surprising fact that we have available only a single thoroughgoing investigation (Lehenbauer's) of the relation of temperature to the growth of higher plants, in spite of the fact that the primary importance of the temperature control of growth is obvious to every observer and has long been qualitatively appreciated. A comparison, for any of the stations employed, of the range of growth values for the plants with the remainder-summation values for temperature (which are practically equivalent to the exponential-summation values in this study) and with the physiological-summation indices will furnish evidence for the verification of these statements. The graphs of the physiological-summation indices of temperature efficiency show much steeper slopes than do the corresponding graphs derived from the other two kinds of temperature indices mentioned above, however, so that the physiological indices are evidently more suitable to represent temperature efficiencies than are either of the other two kinds of indices.

#### LIGHT

The only records of light conditions that were available for all of the stations of this study were the daily ocular estimates of cloudiness obtained by the weather observers, and these alone were used. To make use of these estimates it was, of course, first necessary to bring the daily percentages of clear sky together for each culture period, so as to derive for each period a single value that might be taken to represent the intensity of the light condition for that period. The method employed to accomplish this is presented below.<sup>15</sup>

The total heat equivalent of the actual sunshine for any given period at a given station is primarily a function of three terms: (1) the maximum possible number of hours of sunshine (determined by latitude and season); (2) the mean daily intensity of full sunshine for the period and station, which may be expressed in terms of units of heat received per unit of a horizontal surface; (3) the condition of the sky, whether overcast, partly overcast or clear. The daily values for the first two of these terms vary in a regular manner

<sup>15</sup> The presentation of this method is here practically the same as that previously published. See: Hildebrandt, F. M. A method for approximating sunshine intensity from ocular observations of cloudiness. Johns Hopkins Univ. Circ., March, 1917.

throughout the year for any given place, and the ones for the third term are roughly stated in the observer's records, as just mentioned.

The first two terms are combined in the ordinates of the graph given by Kimball<sup>16</sup> for the maximum possible total radiation received per day at Mount Weather, Virginia. Since this station is at about the same latitude as the stations here dealt with, the ordinates from Kimball's graph may be taken as approximate measures of the total maximum possible light intensities for the corresponding dates for all of the Maryland stations. These values represent the total amount of heat received from the sun and sky on clear days at Mount Weather, in gram-calories per square centimeter of a horizontally exposed surface. The method of using this graph along with the weather observer's reports, for estimating sunshine intensity for any station and period, will be best shown by an example. Suppose it is desired to estimate the average daily sunshine intensity for some station in the general region of Mount Weather, for the first week of August. The average ordinate value for this week is first obtained from Kimball's graph. For periods as short as a week or two this may be done by averaging the values for the first and last days of the period, since the curve may be taken as a straight line for such short intervals. The ordinate values for August 1 and August 7 are ——— and ———, and their average is ———. From the report of the weather observer at the place in question, the number of clear, partly cloudy, and cloudy days is next determined for the days August 1 to August 7, inclusive, and some arbitrary weighting is given to each kind of day. This was done in the present instance by regarding days reported "clear" as days of full sunshine, those reported "partly cloudy" as half days of sunshine, and those reported "cloudy" as without any sunshine. The same scheme of weighting must of course be adhered to in all the estimates used for comparative purposes in any discussion. Suppose there were 2 clear days, 3 half-cloudy days and 2 cloudy days. By summing these values as 2, 1.5 and 0, we obtain 3.5, representing the equivalent number of wholly clear days for the period considered. Now, 3.5 is 0.5 of the total number of days in the week period, and the latter value may be termed "the coefficient of clear weather." By multiplying the average daily intensity value for clear days, ———, (obtained by the use of Kimball's graph) by this coefficient of clear weather (0.5) we obtain ——— g.-cal. as a rough approximation of the average daily sunshine index for the week.

While it is certain that solar radiation affects plants in other ways than through its heating effect, it is no less certain that by far the greater part of the energy of sunshine absorbed by plants is converted into heat (largely as latent heat of vaporization of water), and it seems probable that the other effects produced upon the plant may be more or less proportional to the total

<sup>16</sup> Kimball, Herbert H. The total radiation received on a horizontal surface from the sun and sky at Mount Weather. *Monthly Weather Rev.* 42: 474-487. 1914. (See especially fig. 8, p. 484.)



energy equivalent of sunshine. This method of deriving sunshine indices is, however, to be taken only as a rough approximation.

For each 2-week and for each 4-week period of this study an index of sunshine intensity was secured in the manner just described, and each of these sunshine values was expressed in terms of the average of all values in the series. The relative values thus obtained are quite parallel with the other relative values already referred to. They are given in the tables of data and the actual values may be obtained from them in a manner like that described for the plant values. The general unit used in expressing these relative light values (the average daily sunshine intensity for all stations and for all periods) is 442 gram-calories per square centimeter of horizontal surface.

#### EVAPORATION

The evaporating power of the air was measured as has been said, by means of standardized cylindrical porous-cup atmometers, located so as to have about the same exposure as the plant cultures. The instruments were read at intervals of about two weeks, the dates of reading being the same as those on which observations were made on the plants. After every reading each atmometer cup was removed and replaced by another that had just been standardized. The used cup was subsequently restandardized so as to detect any change in the coefficient of the cup consequent upon its exposure. When the restandardization showed a change in the coefficient, the mean of the original coefficient and the coefficient found upon restandardization was employed to reduce the reading to the Livingston cylindrical standard. The evaporation readings should therefore be directly comparable to other measurements related to the same standard,

As has been pointed out by Livingston,<sup>17</sup> the porous-cup atmometer is somewhat similar to plant foliage in the way in which its evaporating surface is exposed to the surroundings. It may therefore be supposed that the transpiration from the plants for any period should be approximately proportional to the evaporation from the atmometer, *except* in so far as the transpiration rates may be influenced by conditions *within the plant*. The work of Briggs and Shantz indicates that evaporation from small open pans or porous cups is influenced by the same external conditions, and in about the same way, as is plant transpiration, if the comparison is made for periods of a day or more. Of course the two rates do not vary proportionally within the day period, since the internal conditions of the plant exhibit a peculiar daily march, but with such details this study does not need to deal. It has been supposed, therefore, that the effectiveness of the external conditions to influence the transpiration rates of the plants of this study was approxi-

---

<sup>17</sup> Livingston, B. E. The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Wash. Pub. 50. 1906.

mately measured by the corresponding corrected evaporation rates from the atmometer. The atmometer readings have been reduced, in every case, to mean daily rates for the 2-week and 4-week periods, and these rates have been taken as indices of the evaporating power of the air as it affected transpiration from the plants. Finally, all atmometric values have been expressed relatively, in terms of the general average for all stations and for all periods, as in the case of the other data. The general average used as unity for these relative atmometric values is 16.2 cc. per day.

The three derived climatic values in this study may be brought together here, for convenience. The list applies to the 2-week as well as to the 3-week series of data.

(1) *Temperature*. Relative daily mean of the physiological-summation indices for the period.

(2) *Light*. Relative daily mean of calculated light-intensity values for the period, gram-calories per square centimeter of horizontal surface.

(3) *Evaporation*. Relative daily mean of atmometric indices for the period, cubic centimeters of loss from the Livingston standard cylindrical porous cup.

## RESULTS AND DISCUSSION

### INTRODUCTORY

The discussion of the data obtained in this study will be devoted in part to descriptions of the growth changes observed in the plants at the various stations, and for the various periods at each station, and in part to corresponding descriptions of the climatic values. Owing to the complexity of the problem and to the number and variety of the data to be dealt with, it has been found necessary to depart frequently from a general logical order and to treat matters that seem to be of secondary importance at greater length than might appear necessary from a more restricted point of view. Such physiological interpretations as are attempted in the course of the presentation of the data are of interest partly for their own sake, but more particularly because of the bearing they may have on the general problem of the use of standard plants for the comparative integration of effective climatic complexes. The work here reported was planned primarily to make a first trial in the use of standard plants in this way. The discussion of the data will be presented more in the form of a running narrative, with digressions at many points, than is ideally desirable, but the newness of this kind of study and the fact that the fundamental principles and even the terms to be employed have yet to be developed, make anything approaching a true logical sequence quite impossible now.

The various kinds of data to be considered will be brought forward in groups corresponding to their sources. The 2-week plant data and the 2-week cli-

matic data for the stations in the open will first be presented, followed by a presentation of the 4-week plant and climatic data for these stations. Subsequently, a special discussion of the data for the covered stations and a similar treatment of the data for the forest station at Baltimore will be given.

The relative numbers, or indices, derived as described above, are given in tables I to VIII, together with the dates of the first and last days of each culture period and other information, including the length of each period, the number of plants in each culture, etc. Also, a set of figures is presented showing graphically certain parts of the information given in the tables. Tables I to VIII give twenty-six sets of data. Nine of these sets (tables I-III) contain the plant and climatic measurements for the 2-week culture periods for the exposed stations, nine others (tables IV-VI) give the data for the four-week culture periods for the exposed stations, six others (tables VII-VIII) give the data for the 2- and 4-week culture periods for the covered stations at Oakland, Baltimore and Easton, and the two remaining (table VIII) give the data for the 2- and 4-week culture periods for the Baltimore forest station.

In each of the eight tables just mentioned, the first line gives the name of the place referred to, the kind of culture period (whether 2- or 4-week), the character of the exposure of the plants (whether the station is exposed, covered, or forest, and the dates of the beginning and end of each culture period. The second line of each table gives the serial culture numbers. These numbers being assigned to the various cultures for convenience of reference. When several kinds of stations occur at one place, cultures of the same number cover approximately the same time period. For instance, for Baltimore there is an exposed station, a covered station, and a forest station, and there is a 2-week culture "8" for each of these three stations, the dates for each of these being August 20 and September 3. In some cases, culture periods of the same number for the exposed and covered stations show a difference of a day in the lengths of their respective periods owing to the fact that it was impossible to take measurements on both the exposed and covered plants on the same day. The third line of each table gives the length of each culture period, in days. The fourth line gives the number of plants actually used in obtaining the plant measurements. A dash appearing in place of a relative value indicates that the data necessary for calculating this value are lacking. An asterisk placed opposite a climatic index value shows that this particular value was not plotted in the graphs (to be described later). (Points are omitted from the climatic graphs in the case of most cultures where no plant data are available for comparison with the climatic values.) The remainder of the table presents the relative plant and climatic values, the derivation of which has already been made clear. The last column of each of the tables gives the seasonal averages for the station considered.

TABLE I  
Two-week data for exposed stations, Oakland, Chewsville and Monrovia

OAKLAND 2 week periods. EXPOSED STATION.	MAY 23	JUNE 5	JUNE 17	JUNE 29	JULY 10	JULY 21	AUG. 1	AUG. 13	SEPT. 27	SEPT. 12				AV.
	JUNE 5	JUNE 19	JUNE 3	JUNE 15	JULY 26	AUG. 6	AUG. 18	SEPT. 3	SEPT. 15					
	1	2	3	4	5	6	7	8	9					
Culture number	1	2	3	4	5	6	7	8	9					
Length of growing period, days.	13	14	14	15	15	14	13	10	13					
Number of plants	4	5	5	6	5	6	6	6	4					
Remainder summation index.	334	354	401	364	431	369	343	370	282					361
Average daily relative physiological temperature index.	60	66	85	78	87	67	64	66	45					69
Average daily mean temperature, deg F.	65	64	68	67	68	65	66	62	61					65
Average daily relative evaporation index.	153	139	98	79	104	90	71	57	69					96
Average daily relative sunshine intensity.	122	122	109	103	116	102	110	83	81					105
Average daily relative increment in stem height.	76	96	124	84	96	98	101	53	42					80
Average daily relative increment in leaf product.	-	81	67	74	89	70	82	46	-					77

CHEWSVILLE 2 week periods. EXPOSED STATION	MAY 13	JUNE 2	JUNE 10	JUNE 20	JULY 1	JULY 11	AUG. 23	AUG. 31	SEPT. 9	SEPT. 22	OCT. 1			Av
	JUNE 2	JUNE 10	JUNE 20	JUNE 30	JULY 10	AUG. 20	AUG. 28	SEPT. 8	SEPT. 22	OCT. 7	OCT. 20			
	1	2	3	4	5	6	7	8	9	10	11			
Culture number	1	2	3	4	5	6	7	8	9	10	11			
Length of growing period, days.	14	14	14	14	14	14	14	14	14	15	13			
Number of plants	6	6	4	6	5	4	3	5	6	6	6			
Remainder summation index.	416	443	412	453	523	450	517	436	302	328	301			422
Average daily relative physiological temperature index.	92	100	119	112	149	112	145	103	48	51	53			98
Average daily mean temperature, deg F.	69	71	73	71	76	71	76	70	61	61	62			69
Average daily relative evaporation index.	110	115	107	75	108	87	105	80	79	73	64			91
Average daily relative sunshine intensity.	121	121	116	106	121	94	98	67	104	73	44			98
Average daily relative increment in stem height.	93	93	143	112	120	98	10	76	48	37	37			87
Average daily relative increment in leaf product.	104	110	137	90	159	127	100	52	52	20	4			84

MONROVIA 2 week periods. EXPOSED STATION	MAY 18	JUNE 1	JUNE 15	JUNE 29	JULY 13	JULY 27	AUG. 10	AUG. 24	SEPT. 7	SEPT. 21	OCT. 5			AV.
	JUNE 1	JUNE 15	JUNE 29	JULY 13	JULY 27	AUG. 10	AUG. 24	SEPT. 7	SEPT. 21	OCT. 5	OCT. 19			
	1	2	3	4	5	6	7	8	9	10	11			
Culture number	1	2	3	4	5	6	7	8	9	10	11			
Length of growing period, days.	14	14	14	14	14	14	14	14	14	17	11			
Number of plants	3	5	6	4	6	5	6	5	6	6	6			
Remainder summation index.	492	451	478	454	536	465	511	453	384	386	262			437
Average daily relative physiological temperature index.	105	110	124	112	136	117	144	105	59	53	59			104
Average daily mean temperature, deg F.	71	71	73	71	77	72	73	70	62	62	63			70
Average daily relative evaporation index.	-	146	152	93	117	120	112	68	96	82	76			106
Average daily relative sunshine intensity.	132	122	104	102	110	105	98	82	103	66	52			100
Average daily relative increment in stem height.	73	81	121	96	115	90	101	76	20	67	25			79
Average daily relative increment in leaf product.	66	90	110	83	138	109	104	112	6	56	4			80

TABLE II  
Two-week data for exposed stations, College, Baltimore and Darlington

COLLEGE 2-week periods: EXPOSED STATION	MAY 9	MAY 10	JUNE 11	JUNE 12	JULY 13	JULY 14	AUG. 15	AUG. 16	SEPT. 17	SEPT. 18	OCT. 19	OCT. 20	AV.
Culture number	2	3	4	5	6	7	8	9	10	11	12		
Length of growing period, days	14	13	14	14	14	14	13	14	15	15	14		
Number of plants	6	5	5	6	4	4	5	6	-	6	6		
Remainder summation index	546	104	576	494	500	194	118	111	570	555	521		450
Average daily relative physiological temperature index	108	103	157	154	148	133	143	115	57	50	51		108
Average daily mean temperature, deg. F	71	70	76	74	75	74	76	71	84	81	84		75
Average daily relative evaporation index	100	154	123	96	143	141	135	127	73	54	55		117
Average daily relative sunshine intensity	-	-	-	-	-	-	-	-	-	-	-		
Average daily relative increment in stem height	93	152	115	118	124	116	90	76	-	48	53		95
Average daily relative increment in leaf-product	96	123	81	152	203	150	117	102	-	13	10		121

BALTIMORE 2-week periods: EXPOSED STATION	MAY 13	MAY 14	JUNE 15	JUNE 16	JULY 17	JULY 18	AUG. 19	AUG. 20	SEPT. 21	SEPT. 22	OCT. 23	OCT. 24	AV.
Culture number	1	2	3	4	5	6	7	8	9	10	11		
Length of growing period, days	13	12	13	14	14	14	14	14	15	12	13		
Number of plants	3	-	5	6	6	4	5	5	6	5	5		
Remainder summation index	151	310	508	489	540	506	511	502	317	500	503		448
Average daily relative physiological temperature index	31	31	103	105	102	112	100	127	62	97	95		112
Average daily mean temperature, deg. F	67	70	73	73	73	75	75	73	64	64	64		72
Average daily relative evaporation index	127	105	115	80	12	112	110	81	59	58	64		85
Average daily relative sunshine intensity	137	84	108	60	94	72	79	74	82	59	59		76
Average daily relative increment in stem height	93	-	146	152	183	152	152	147	75	56	70		125
Average daily relative increment in leaf-product	106	-	128	163	194	110	233	130	54	22	47		119

DARLINGTON 2-week periods: EXPOSED STATION	MAY 15	MAY 16	JUNE 17	JUNE 18	JULY 19	JULY 20	AUG. 21	AUG. 22	SEPT. 23	SEPT. 24	OCT. 25	OCT. 26	AV.
Culture number	1	2	3	4	5	6	7	8	9	10	11		
Length of growing period, days	15	14	13	14	14	14	14	14	14	14	14		
Number of plants	6	5	6	4	5	5	-	5	6	5	4		
Remainder summation index	440	432	596	456	542	450	506	422	439	515	438		416
Average daily relative physiological temperature index	81	99	94	93	113	113	146	123	49	87	54		111
Average daily mean temperature, deg. F	68	70	69	70	76	75	76	75	61	62	65		69
Average daily relative evaporation index	135	123	90	67	74	77	64	53	32	37	34		87
Average daily relative sunshine intensity	154	129	92	77	111	111	116	79	56	115	47		100
Average daily relative increment in stem height	110	104	115	141	111	110	-	127	42	113	57		115
Average daily relative increment in leaf-product	135	129	150	112	111	140	-	118	29	113	11		116

TABLE III

*Two-week data for exposed stations, Coleman, Easton and Princess Anne*

COLEMAN 2-week periods EXPOSED STATION	MAY 15	MAY 25	JUNE 11	JUNE 21	JULY 5	JULY 15	AUG 3	AUG 13	SEPT 1	SEPT 11	SEPT 21	OCT 1	AV.
	MAY 25	JUNE 11	JUNE 21	JULY 5	JULY 15	AUG 3	AUG 13	SEPT 1	SEPT 11	SEPT 21	OCT 1	OCT 11	
	25	11	21	5	15	3	13	1	11	21	1	11	
Culture number	1	2	3	4	5	6	7	8	9	10	11	12	
Length of growing period, days	15	14	13	14	14	14	14	14	14	14	13	15	
Number of plants	6	6	4	4	6	6	5	6	4	4	4	6	
Remainder summation index	412	465	435	488	560	517	535	520	510	597	—	—	472
Average daily relative physiological temperature index	81	113	118	130	170	145	158	148	84	85	—	—	125
Average daily mean temperature, deg. F.	66	72	67	74	79	76	77	76	61	67	—	—	72
Average daily relative evaporation index	154	152	127	120	143	155	119	96	132	135	7	93	127
Average daily relative sunshine intensity	142	100	125	61	141	124	118	85	—	—	—	—	120
Average daily relative increment in stem height	79	81	101	135	149	157	121	107	62	53	67	28	96
Average daily relative increment in leaf product	108	111	97	172	211	196	158	110	33	53	38	4	107

EASTON 2-week periods EXPOSED STATION	MAY 8	MAY 25	JUNE 8	JUNE 22	JULY 6	JULY 20	AUG 3	AUG 17	AUG 31	SEPT 14	SEPT 28	OCT 11	AV.
	MAY 25	JUNE 8	JUNE 22	JULY 6	JULY 20	AUG 3	AUG 17	SEPT 1	SEPT 15	SEPT 29	OCT 13	OCT 26	
	25	8	22	6	20	3	17	1	15	29	13	26	
Culture number	1	2	3	4	5	6	7	8	9	10	11	12	
Length of growing period, days	17	14	14	14	14	14	14	14	14	13	15	15	
Number of plants	6	4	5	6	4	5	5	5	5	6	6	5	
Remainder summation index	430	455	451	492	532	495	515	519	592	381	320	347	445
Average daily relative physiological temperature index	62	112	110	131	154	153	144	148	87	79	64	42	105
Average daily mean temperature, deg. F.	65	71	71	74	77	74	76	76	67	66	64	62	71
Average daily relative evaporation index	95	130	133	91	86	123	128	113	111	134	88	82	103
Average daily relative sunshine intensity	145	112	141	120	124	108	117	77	97	53	64	52	111
Average daily relative increment in stem height	43	67	112	132	140	146	152	118	81	43	65	54	75
Average daily relative increment in leaf product	71	111	112	99	152	130	163	129	64	33	—	—	108

PRINCESS ANNE 2-week periods EXPOSED STATION	MAY 11	MAY 25	JUNE 8	JUNE 23	JULY 7	JULY 21	AUG 4	AUG 18	SEPT 1	SEPT 15	SEPT 29	OCT 12	AV.
	MAY 25	JUNE 8	JUNE 23	JULY 7	JULY 21	AUG 4	AUG 18	SEPT 1	SEPT 15	SEPT 29	OCT 12	OCT 27	
	25	8	23	7	21	4	18	1	15	29	12	27	
Culture number	1	2	3	4	5	6	7	8	9	10	11	12	
Length of growing period, days	15	15	15	14	14	14	14	14	14	14	13	15	
Number of plants	6	5	6	6	6	6	5	6	6	5	5	4	
Remainder summation index	359	409	504	443	514	485	448	525	387	365	515	523	432
Average daily relative physiological temperature index	81	105	109	132	144	133	144	146	84	71	60	43	101
Average daily mean temperature, deg. F.	62	70	73	74	76	74	76	76	67	67	63	61	70
Average daily relative evaporation index	117	136	—	—	63	95	101	74	72	60	39	34	
Average daily relative sunshine intensity	107	110	103	57	83	70	73	85	64	56	67	42	86
Average daily relative increment in stem height	67	110	104	124	141	143	155	134	79	65	53	45	106
Average daily relative increment in leaf product	36	110	104	115	163	160	177	135	54	34	18	6	96

TABLE IV  
Four-week data for exposed stations, Oakland, Chewsville and Monrovia

OAKLAND 4-week periods. EXPOSED STATION		MAY 22	JUNE 5	JUNE 19	JUNE 3	JUNE 18	JUNE 31	AUG. 14	AUG. 27	SEPT. 10	SEPT. 23	SEPT. 6	SEPT. 19	SEPT. 3	SEPT. 16	SEPT. 29	SEPT. 12	SEPT. 25	Av.
Culture number		1	2	3	4	5	6	7	8										
Length of growing period, days.		27	29	27	28	29	27	29	29										
Number of plants		4	5	5	6	5	6	6	6										
Remainder summation index.		683	725	765	776	800	813	764	652										735
Average daily relative physiological temperature index.		68	76	82	83	77	86	65	55										71
Average daily mean temperature, deg F.		68	66	67	67	67	68	64	62										65
Average daily relative evaporation index.		146	119	89	92	97	81	64	63										94
Average daily relative sunshine intensity.		122	116	106	110	109	106	97	82										106
Average daily relative increment in stem height.		66	73	94	69	72	81	63	47										71
Average daily relative increment in leaf area.		41	67	91	75	80	90	73	52										71
Average daily relative increment in dry weight.		21	71	103	83	80	83	76	46										79

CHEWSVILLE 4-week periods. EXPOSED STATION		MAY 19	JUNE 2	JUNE 16	JUNE 30	JUNE 14	JULY 28	AUG. 11	AUG. 25	SEPT. 8	SEPT. 22	SEPT. 6	SEPT. 20	OCT. 4	OCT. 18	OCT. 31	NOV. 14	NOV. 27	Av.
Culture number		1	2	3	4	5	6	7	8	9	10	11							
Length of growing period, days.		26	28	28	28	28	28	28	28	29	28	27							
Number of plants.		6	6	4	6	5	4	3	3	6	6	6							
Remainder summation index.		899	915	925	976	973	967	953	736	630	629	490							823
Average daily relative physiological temperature index.		99	113	116	131	131	129	124	76	50	52	37							96
Average daily mean temperature, deg F.		73	72	72	74	74	74	73	66	61	62	58							69
Average daily relative evaporation index.		113	111	91	92	98	96	93	80	76	69	71							90
Average daily relative sunshine intensity.		125	123	111	114	108	96	83	86	69	59	56							95
Average daily relative increment in stem height.		73	103	122	97	91	84	84	50	47	41	31							75
Average daily relative increment in leaf area.		73	91	132	83	89	94	99	57	44	45	12							74
Average daily relative increment in dry weight.		70	102	142	87	92	86	86	62	51	38	24							78

MONROVIA 4-week periods. EXPOSED STATION		MAY 12	JUNE 1	JUNE 15	JUNE 29	JULY 13	JULY 27	AUG. 10	AUG. 24	SEPT. 7	SEPT. 21	SEPT. 5	SEPT. 19	OCT. 3	OCT. 17	OCT. 31	NOV. 14	NOV. 28	Av.
Culture number		1	2	3	4	5	6	7	8	9	10	11							
Length of growing period, days.		28	28	28	28	28	28	28	28	31	28	25							
Number of plants.		3	5	6	4	6	5	6	5	6	6	6							
Remainder summation index.		896	932	932	990	997	974	944	817	770	646	463							851
Average daily relative physiological temperature index.		108	117	118	134	137	131	125	82	56	56	53							102
Average daily mean temperature, deg F.		71	72	72	74	75	74	73	66	62	61	58							69
Average daily relative evaporation index.		149	123	103	119	116	90	82	89	79	61								103
Average daily relative sunshine intensity.		131	113	103	106	108	102	90	94	86	59	58							96
Average daily relative increment in stem height.		63	78	84	84	78	91	84	56	44	41	28							66
Average daily relative increment in leaf area.		71	108	88	99	71	104	75	70	39	48	12							71
Average daily relative increment in dry weight.		70	114	108	99	89	103	81	87	41	48	24							79

TABLE V  
Four-week data for exposed stations, College, Baltimore and Darlington

COLLEGE 4 week periods EXPOSED STATION	MAY 9 JUNE 6	MAY 23 JUNE 20	JUNE 6 JULY 3	JUNE 13 JULY 10	JULY 3 JULY 31	JULY 17 AUG. 14	JULY 31 AUG. 27	AUG. 14 SEPT. 10	AUG. 27 SEPT. 24	SEPT. 10 OCT. 8	SEPT. 24 OCT. 21	Av.
Culture number.	1	2	3	4	5	6	7	8	9	10	11	
Length of growing period, days.	26	27	27	28	28	28	27	27	29	30	29	
Number of plants.	6	6	5	5	6	4	4	6	6	—	6	
Remainder summation index.	888	750	750	1020	1014	1074	712	727	627	713	662	902
Average daily relative physiological temperature index.	88	110	121	137	141	141	136	127	91	57	52	116
Average daily mean temperature, deg. F.	67	71	73	75	75	75	75	74	69	65	62	71
Average daily relative evaporation index.	109	157	139	110	120	145	141	121	93	81	83	124
Average daily relative sunshine intensity.	—	—	—	—	—	—	—	—	—	—	—	
Average daily relative increment in stem height.	47	75	100	91	91	97	103	72	63	—	56	80
Average daily relative increment in leaf area.	70	76	125	73	98	173	207	126	86	—	46	110
Average daily relative increment in dry weight.	86	110	140	135	117	172	199	132	84	—	51	119

BALTIMORE 4 week periods EXPOSED STATION	MAY 14 JUNE 10	MAY 28 JUNE 25	JUNE 10 JULY 7	JUNE 17 JULY 14	JULY 7 AUG. 4	AUG. 4 AUG. 20	AUG. 20 SEPT. 17	SEPT. 17 OCT. 14	SEPT. 30 OCT. 27	OCT. 14 OCT. 31	Av.	
Culture number.	1	2	3	4	5	6	7	8	9	10	11	
Length of growing period, days.	27	27	28	28	28	28	28	28	28	28	28	
Number of plants.	4	5	5	6	6	4	6	5	6	5	5	
Remainder summation index.	82	818	997	632	652	649	1042	697	670	619	619	805
Average daily relative physiological temperature index.	72	111	124	144	151	150	150	101	65	47		110
Average daily mean temperature, deg. F.	70	70	73	75	77	77	77	69	64	60		71
Average daily relative evaporation index.	118	112	113	96	102	111	96	73	69	66	61	90
Average daily relative sunshine intensity.	113	111	104	90	86	70	77	73	66	74	50	82
Average daily relative increment in stem height.	100	114	139	122	122	131	124	91	64	75	56	104
Average daily relative increment in leaf area.	90	107	115	147	134	141	196	97	75	79	51	115
Average daily relative increment in dry weight.	80	112	119	136	124	116	162	72	59	67	45	105

DARLINGTON 4 week periods EXPOSED STATION	MAY 13 JUNE 10	MAY 27 JUNE 24	JUNE 10 JULY 7	JUNE 17 JULY 14	JULY 7 AUG. 4	AUG. 4 AUG. 21	AUG. 21 SEPT. 18	SEPT. 18 OCT. 15	SEPT. 30 OCT. 27	Av.	
Culture number.	1	2	3	4	5	6	7	8	9	10	
Length of growing period, days.	29	27	27	28	28	28	28	28	28	28	
Number of plants.	6	5	6	4	5	5	—	5	6	5	
Remainder summation index.	902	804	664	604	682	702	708	1004	664	683	842
Average daily relative physiological temperature index.	93	77	72	124	130	130	—	123	57		100
Average daily mean temperature, deg. F.	71	70	70	73	74	75	75	67	67	63	70
Average daily relative evaporation index.	128	106	76	69	79	83	—	123	86	117	85
Average daily relative sunshine intensity.	142	111	65	73	60	64	61	133	71	70	71
Average daily relative increment in stem height.	122	134	111	117	172	131	—	100	71	100	123
Average daily relative increment in leaf area.	156	107	101	100	150	162	—	100	91	94	148
Average daily relative increment in dry weight.	124	100	75	100	164	160	—	89	70	72	94

TABLE VI

*Four-week data for exposed stations, Coleman, Easton and Princess Anne*

COLLEMAN 4-week periods EXPOSED STATION	MAY 15	MAY 20	JUNE 11	JUNE 24	JULY 8	JULY 22	AUG. 5	AUG. 19	SEPT. 2	SEPT. 16	SEPT. 30	Av.
	MAY 11	MAY 24	JUNE 8	JUNE 22	JULY 6	AUG. 20	SEPT. 3	SEPT. 17	OCT. 1	OCT. 15	OCT. 29	
Culture number	1	2	5	4	2	6	7	8	9	10	11	
Length of growing period, days	77	71	74	70	69	68	69	69	69	67	68	
Number of plants	6	6	4	4	6	6	5	5	5	4	5	
Remainder summation index	818	879	721	698	1011	1055	1050	1100	1000	-	-	109
Average daily relative physiological temperature index	91	116	124	150	158	150	155	110	85	-	-	229
Average daily mean temperature, deg. f	69	75	71	71	78	77	77	72	61			73
Average daily relative evaporation index	146	145	121	130	142	137	100	113	134	116	15	125
Average daily relative sunshine intensity	151	143	95	101	155	121	102	-	-	-	-	121
Average daily relative increment in stem height	69	81	94	100	100	107	81	72	58	50	55	87
Average daily relative increment in leaf area	116	150	161	164	155	145	108	89	71		41	117
Average daily relative increment in dry weight	126	155	159	143	134	148	83	113	97	75	43	115

EASTON 4-week periods EXPOSED STATION	MAY 15	MAY 20	JUNE 8	JUNE 24	JULY 8	JULY 22	AUG. 5	AUG. 19	SEPT. 2	SEPT. 16	SEPT. 30	Av.
	MAY 11	MAY 24	JUNE 8	JUNE 22	JULY 6	AUG. 20	SEPT. 3	SEPT. 17	OCT. 1	OCT. 15	OCT. 29	
Culture number	1	2	5	4	2	6	7	8	9	10	11	
Length of growing period, days	71	68	70	70	70	70	70	70	71	70	70	
Number of plants	6	4	-	6	4	5	5	5	5	6	5	
Remainder summation index	889	104	945	1024	1027	1008	1030	1111	115	707	675	100
Average daily relative physiological temperature index	70	111	121	145	144	137	145	117	90	70	55	105
Average daily mean temperature, deg. f	68	71	75	76	76	75	75	72	67	66	63	70
Average daily relative evaporation index	115	122	110	87	110	131	127	112	103	76	65	107
Average daily relative sunshine intensity	157	157	151	122	110	115	105	78	70	50	58	128
Average daily relative increment in stem height	47	72	71	103	105	115	103	81	50	50	50	75
Average daily relative increment in leaf area	74	76	111	121	121	121	121	75	60	64	54	92
Average daily relative increment in dry weight	80	81	111	91	115	121	121	81	72	48	42	85

PRINCESS ANNE 4-week periods EXPOSED STATION	MAY 15	MAY 20	JUNE 8	JUNE 24	JULY 8	JULY 22	AUG. 5	AUG. 19	SEPT. 2	SEPT. 16	SEPT. 30	Av.
	MAY 11	MAY 24	JUNE 8	JUNE 22	JULY 6	AUG. 20	SEPT. 3	SEPT. 17	OCT. 1	OCT. 15	OCT. 29	
Culture number	1	2	5	4	5	6	7	8	9	10	11	
Length of growing period, days	78	78	79	78	78	78	78	78	78	77	78	
Number of plants	6	5	5	6	6	6	5	6	6	5	6	
Remainder summation index	100	109	112	1007	111	110	1095	101	120	108	615	100
Average daily relative physiological temperature index	80	102	117	136	136	136	145	110	78	66	50	120
Average daily mean temperature, deg. f	67	71	75	75	75	75	70	72	68	64	60	71
Average daily relative evaporation index	126	-	-	-	75	92	70	78	78	61	55	85
Average daily relative sunshine intensity	115	111	94	87	92	20	21	25	61	15	55	87
Average daily relative increment in stem height	65	80	100	100	100	100	100	100	100	100	100	100
Average daily relative increment in leaf area	125	100	100	-	111	130	120	100	100	100	100	117
Average daily relative increment in dry weight	130	100	137	110	111	100	71	111	100	100	100	100

TABLE VII  
Data for covered stations, Oakland and Baltimore

OAKLAND 2-week periods. COVERED STATION.	MAY 22	JUNE 4	JUNE 18	JULY 2	JULY 15	JULY 30	AUG. 13	AUG. 26	SEPT. 11							AV.
	JUNE 4	JUNE 18	JULY 2	JULY 15	JULY 30	AUG. 13	AUG. 26	SEPT. 11	SEPT. 23							
Culture number.	1	2	3	4	5	6	7	8	9							
Length of growing period, days.	13	14	14	15	15	14	13	16	13							
Number of plants.	5	6	6	5	6	—	5	5	5							
Average daily relative evaporation index.	112	147	133	109	117	112	112	85	93							120
Average daily relative increment in stem height.	56	112	110	121	126	—	84	70	56							92
Average daily relative increment in leaf-product.	18	85	127	96	154	—	87	44	9							78

OAKLAND 4-week periods. COVERED STATION.	MAY 22	JUNE 4	JUNE 18	JULY 2	JULY 15	JULY 30	AUG. 13	AUG. 26	SEPT. 11	SEPT. 23						AV.
	JUNE 18	JULY 2	JULY 15	JULY 30	AUG. 13	AUG. 26	SEPT. 11	SEPT. 23								
Culture number.	1	2	3	4	5	6	7	8								
Length of growing period, days.	27	28	27	28	29	27	29	29								
Number of plants.	5	6	6	5	6	—	5	5								
Average daily relative evaporation index.	160	140	121	113	115	112	99	79								117
Average daily relative increment in stem height.	56	91	105	106	97	—	63	66								81
Average daily relative increment in leaf area.	—	107	145	155	118	—	—	66								118
Average daily relative increment in dry weight.	57	97	121	123	111	—	86	59								93

BALTIMORE 2-week periods. COVERED STATION.			JUNE 10	JUNE 25	JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	SEPT. 1	OCT. 1				AV.
			JUNE 25	JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1	OCT. 14					
Culture number.			3	4	5	6	7	8	9	10	11					
Length of growing period, days.			15	14	14	14	14	14	16	12	13					
Number of plants.			6	6	4	6	5	5	3	5	4					
Average daily relative evaporation index.			119	95	112	109	117	87	81	77	73					97
Average daily relative increment in stem height.			157	160	326	253	197	191	56	70	155					172
Average daily relative increment in leaf-product.			111	150	262	147	180	163	51	21	43					125

BALTIMORE 4-week periods. COVERED STATION.	MAY 29	JUNE 10	JUNE 25	JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1						AV.
	JUNE 25	JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1	OCT. 14	OCT. 31						
Culture number.	2	3	4	5	6	7	8	9	10	11						
Length of growing period, days.	27	29	28	28	28	28	30	28	25	30						
Number of plants.	5	6	6	4	6	5	5	3	5	4						
Average daily relative evaporation index.	119	107	107	111	113	102	84	79	75	7						97
Average daily relative increment in stem height.	156	128	154	231	169	153	131	72	103	73						150
Average daily relative increment in leaf area.	—	—	188	250	131	171	124	74	101							154
Average daily relative increment in dry weight.	84	54	116	180	103	153	114	64	60	57						95

TABLE VIII

*Data for covered station at Easton and for forest station at Baltimore*

EASTON 2-week periods. COVERED STATION.		MAY 25	JUNE 8	JUNE 22	JULY 6	JULY 20	AUG. 3	AUG. 17	AUG. 31	SEPT. 14	SEPT. 28		Av.
		JUNE 8	JUNE 22	JULY 6	JULY 20	AUG. 3	AUG. 17	SEPT. 14	SEPT. 28	OCT. 11			
Culture number		2	3	4	5	6	7	8	9	10	11		
Length of growing period, days.		14	14	14	14	14	14	14	14	14	14		
Number of plants.		4	6	4	3	6	6	5	6	6	6		
Average daily relative evaporation index.		166	135	86	112	144	129	131	131	104	103		124
Average daily relative increment in stem height.		129	118	155	177	177	177	185	96	90	—		145
Average daily relative increment in leaf-product.		122	112	89	100	162	210	225	125	55	—		142

EASTON 4-week periods. COVERED STATION.		MAY 25	JUNE 8	JUNE 22	JULY 6	JULY 20	AUG. 3	AUG. 17	AUG. 31	SEPT. 14	SEPT. 28	OCT. 11	Av.
		JUNE 22	JULY 6	JULY 20	AUG. 3	AUG. 17	SEPT. 14	SEPT. 28	OCT. 11	OCT. 26	NOV. 9		
Culture number		2	3	4	5	6	7	8	9	10	11	12	
Length of growing period, days.		28	28	28	28	28	28	28	28	27	28	26	
Number of plants.		4	6	6	—	6	6	5	6	6	—	—	
Average daily relative evaporation index.		151	108	96	128	137	130	131	118	103	124	116	120
Average daily relative increment in stem height.		103	128	125	—	134	150	125	75	81	—	—	116
Average daily relative increment in leaf area.		117	191	90	—	138	202	169	104	85	—	—	137
Average daily relative increment in dry weight.		108	144	86	—	107	140	129	105	56	—	—	109

BALTIMORE 2-week periods. FOREST STATION.				JUNE 21	JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1		Av.
				JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1	OCT. 14		
Culture number				4	5	6	7	8	9	10	11		
Length of growing period, days.				18	14	14	14	14	16	12	13		
Number of plants.				6	5	—	4	5	4	6	3		
Average daily relative evaporation index.				—	75	82	67	72	64	57	52		67
Average daily relative increment in stem height.				261	458	—	576	284	171	101	208		271
Average daily relative increment in leaf-product.				94	104	—	130	59	15	6	21		52

BALTIMORE 4-week periods. FOREST STATION.				JUN. 21	JULY 9	JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1		Av.
				JULY 23	AUG. 6	AUG. 20	SEPT. 3	SEPT. 19	OCT. 1	OCT. 14	OCT. 28		
Culture number				4	5	6	7	8	9	10	11		
Length of growing period, days.				32	28	28	28	30	28	25	30		
Number of plants.				6	5	—	4	5	5	6	3		
Average daily relative evaporation index.				—	79	75	70	60	61	55	55		66
Average daily relative increment in stem height.				309	444	—	510	215	200	200	126		259
Average daily relative increment in leaf area.				104	104	—	95	75	40	58	32		69
Average daily relative increment in dry weight.				52	62	—	46	57	50	53	27		41

The 2-week tables for the exposed stations (tables I-III) show in line 5 the remainder-summation temperature index for each culture period, this being obtained by subtracting  $39^{\circ}$  from each daily mean and then summing the remainders for the period. Line 6 gives the average daily relative physiological index for each period. Line 7 gives the average daily mean temperatures for each period, line 8 the average daily relative evaporation index, and line 9, the average daily relative sunshine-intensity value. Line 10 shows the values of the average daily relative increment of stem height, and line 11 the values of the average daily relative increment of leaf-product. The two-week tables for the covered stations correspond to the two-week tables for the exposed stations, except that no temperature or sunshine data are here available and the tables thus contain only the relative evaporation indices and the two plant values. This is also true for the Baltimore forest station.

The 4-week tables correspond, line for line, with the 2-week ones, except that the 4-week tables show the average daily relative increment of leaf area (instead of the average daily relative increment of leaf-product) and a line is added to the 4-week tables giving the average daily relative increment of dry weight. Each 4-week value of the relative daily physiological temperature index, the relative daily evaporation index, and the relative daily sunshine intensity, was obtained by averaging the relative values of these climatic factors for the 2-week periods in question. The 4-week value of the remainder-summation index for each period was obtained by adding the values of this index for the two 2-week periods that make up the 4-week period under consideration. The average daily mean temperature for the longer periods was obtained by taking the mean of the two average daily means for the two 2-week periods involved.

It will be noted that the plant values are uniformly given at the bottom of the table, with a double rule separating them from what precedes.

Figures 2-6 present graphically certain of the data given in tables I-VIII. Graphs for plant values are denoted by black lines and those for climatic values are shown in red. In all of these graphs the ordinates represent magnitudes of the plant and climatic relative values and the abscissas represent the time of the year. The ordinate scale is given at the left of each set of graphs, for convenience of reference, and the dates of the beginnings of successive culture periods are shown on the base line. Thus, for the first 2-week period at Oakland, the ordinates show the average daily relative values of the plant and climatic measurements for the 2-week period beginning May 23. The 100-line of the ordinate scale represents the average seasonal value for all stations (as previously noted), this being the unit used in expressing the corresponding relative values. Full black lines (appearing only on 4-week graphs) represent dry weight. Dash black lines represent height. Dotted black lines (only on 4-week graphs) represent leaf area.

Dash-and-dot lines (only on 2-week graphs) represent leaf-product. Full red lines represent temperature. Dash red lines represent evaporation. Dotted red lines represent light.

The results obtained will now be brought forward, with some discussion, which is to be read with reference to tables I-VIII and figures 2-6.

#### RESULTS FOR STATIONS IN THE OPEN

The data for the stations in the open will be considered as of two main groups, *the 2-week data* and *the 4-week data*.

##### THE 2-WEEK VALUES

*The 2-week plant data for stations in the open (see figs. 2 and 3, black lines)*

As has been stated, the plant measurements here in question were taken about two weeks after planting and included stem height and leaflet dimensions. From these have been derived (1) the relative mean daily rate of increase in stem height per plant and (2) the relative mean daily rate of increase in total leaf-product per plant, both for each 2-week period.

Therefore, one of these 2-week plant values represents the stem-producing power of the plant and the other stands for its leaf-producing power, under the given set of external conditions acting during that period. Since the plants are taken to be alike at the start, (seeds) these two derived plant values should be the same for all individuals if all were subjected to the same effective environmental conditions throughout the period, and when the various plants are exposed to different environments the values just mentioned become criteria by which the effectiveness of one environment may be compared with that of another, with reference, of course, to the particular set of internal conditions represented by the plants at the beginning of the tests. The two plant values just mentioned may thus be regarded as relative measures of the effectiveness or efficiency of the environmental complex for the 2-week period considered, as it acted to produce stem elongation and leaf-product increase, upon the soy-bean plants employed in this investigation. For convenience, the following discussion will refer to the graphs (figs. 2 and 3) rather than to the tables, but tables and graphs both present the same data in every case. This discussion will be given under two headings: (1) Correlations between the two plant graphs and (2) Trend of the plant values and their seasonal averages for the various stations.

*Correlations between the two 2-week plant graphs.*—It is readily seen that the two graphs showing relative rates of increase in stem height and in leaf-product agree in their general direction of slope from period to period, throughout the season and for all stations. In many cases the two plant graphs not only slope in the same general direction (upward or downward) but their

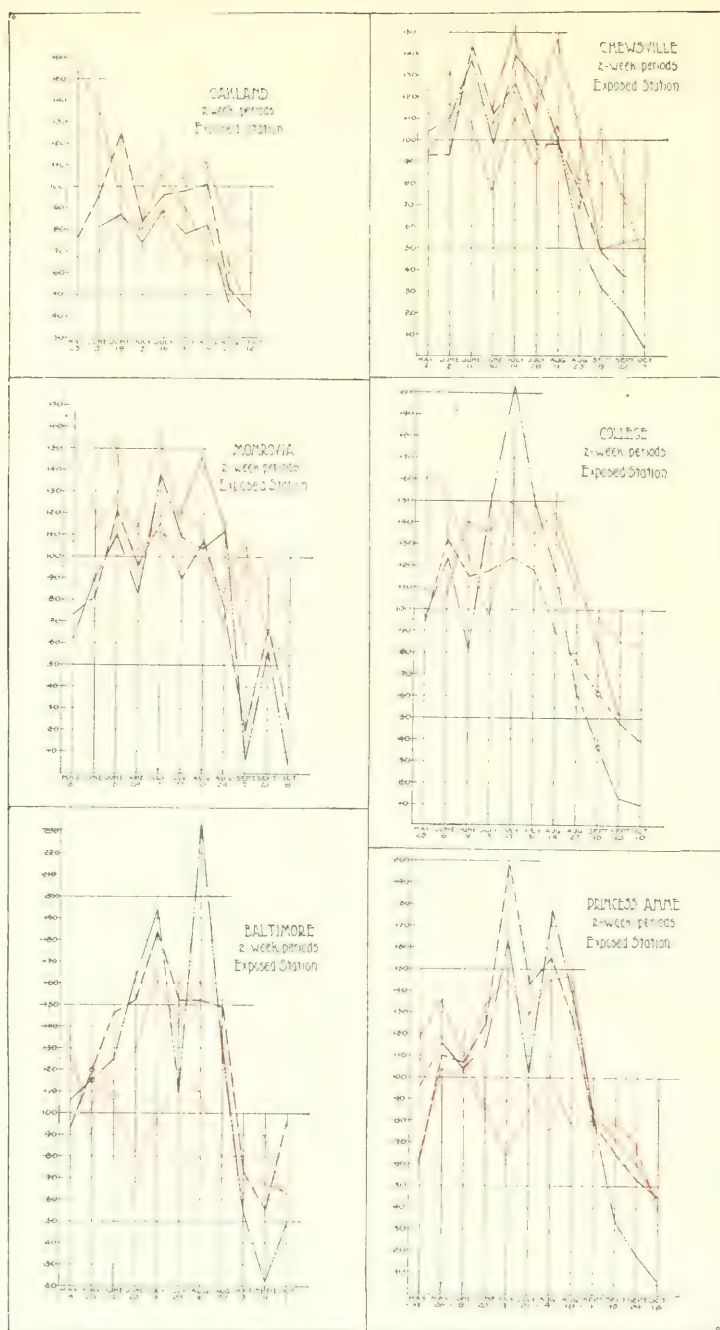


FIG. 2. Graphs of 2-week data for exposed stations, as named.

Black: Height, — — — —; Leaf product, — . . . — . . .

Red: Temperature index, —————; Evaporation index, — — — —; Sunlight index, . . . . .

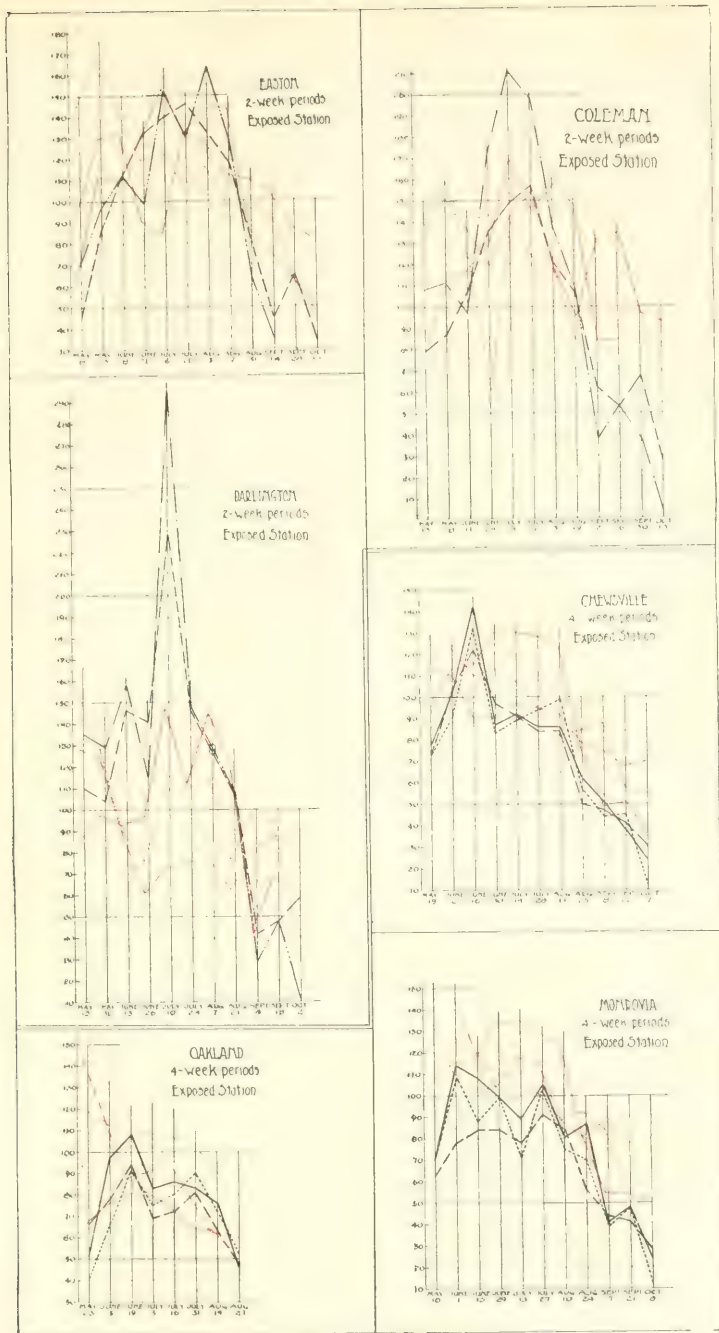


FIG. 3. Graphs of 2-week data for exposed stations, as named (continued).  
 (Lines as in fig. 2.)—Graphs of 4-week data for exposed stations, as named.  
 Black: Dry weight, ————; Height, — — — —; Leaf area; .....  
 Red, as in fig. 2.

corresponding angles of slope are nearly the same and their corresponding ordinates are about equal, so that they nearly coincide for considerable portions of their length. In other words, there appears to have been a pronounced general agreement between the effectiveness of the environment to produce stem elongation and its effectiveness to increase the magnitude of the leaf-product, as shown by these cultures. If this agreement were perfect it would mean, of course, that the environment exerted the same influence upon the process of leaf-surface increase (as measured by leaf-product) and upon the process of stem elongation, and either of these two criteria would be a measure of the other. But the coincidence of the two graphs is not by any means perfect and it becomes a matter of interest to study their differences, as shown by the corresponding relative values of their ordinates.

Inspection of the graphs shows that, leaving those for Oakland out of account, the index for stem height increase is frequently greater than the other plant index for the early and late portions of the frostless season, and that this relation generally is reversed for the middle portion. In other terms, the graph for stem elongation generally lies below the other graph for the middle of the season and above it for the beginning and end of the season. In still other words, the seasonal maxima of leaf-product values are generally relatively higher than those of stem elongation, while the seasonal minima of the former are lower than those of the latter. It may be stated, as an approximation, that when these two plant values are both about 100 (as the data are presented in this paper) the leaf-product value is generally the higher of the two, while when both are below 100 the elongation value is usually the higher. In the case of Oakland, both values are comparatively very low throughout the season and, while the index of stem elongation reaches somewhat above 100 for two periods, this index is never surpassed in magnitude by the index of leaf-product increase.

The generalization just stated indicating a relation between the rates of two plant processes, seems to be a physiological one, dependent upon the nature of the soy-bean plant and hence largely predetermined by the internal conditions of the seed. Within the range of environmental conditions encountered in this study it appears that the taller and more leafy the plant becomes in the first two weeks of growth, the lower is the value of the ratio of final height to final foliar expanse. The two growth processes here considered are, therefore, clearly interrelated and neither one alone is to be regarded as a criterion of plant growth in general. The average of these two indices may be considered as a tentative index of the general growth of the plants during the first two weeks from the seed. Inspection of the 2-week graphs leads to the impression that this mean of the two values offers perhaps the most promising way to obtain from them a single index of plant growth. The two are always so nearly parallel throughout the season (nearly coinciding for many periods, as has been stated) that the charts have not been further

complicated by introducing the graph for the average, but the form of this graph is readily appreciated from the two graphs that are given.

The general relation between the two plant values that has just been emphasized does not always hold, and the more detailed discussion of the plant graphs for individual stations, given in the next following paragraph, is of value in showing the main exceptions.

For Oakland the height value lies above that for leaf-product throughout the season. The two graphs have the same general direction of slope except for the period beginning July 16. For Chewsville the height graph exhibits the same general direction of slope as does the leaf-product graph, from period to period, throughout the season, with the former well above the latter for the last four periods (beginning August 25, September 8, September 22 and October 27). For the periods beginning June 16 and June 30 the latter relation also holds, although the index values are large, especially in the case of the first of these two periods. For Monrovia the two plant graphs follow each other very closely throughout the entire season. The graphs for College for the periods beginning July 3, July 17 and July 31, illustrated the tendency of the height values to decrease relatively to those of leaf-product when both values are large. For the periods beginning September 10, September 25 and October 10 for this station, both values are small and, as would be expected, stem height is relatively greater than leaf product. For the period beginning June 19 the expected relation between the two graphs does not obtain. For Baltimore the periods beginning June 10, July 23 and August 20 are exceptions to the proposition that the height graph should lie below the graph of leaf-product when both plant values are large. The generalization is true, however, for the remaining periods of the Baltimore season. For Darlington the two plant graphs agree closely in value throughout the season. For Coleman stem height and leaf-product show the expected relation. For Easton the generalization holds, with three exceptions: the height index is lower than the other for the period beginning May 8, although both indices have low values, and this relation is reversed for the periods beginning June 22 and July 20, in spite of the fact that both values are large in these cases. For Princess Anne the graphs show values of the height index higher than those of the other index for the periods beginning June 23, July 7 and July 21, although both indices are large for all three periods. Otherwise these graphs agree with the generalization.

The fact that the generalization given above holds in the great majority of the cases here studied renders the exceptions of special interest. Assuming that the seeds were all alike at the beginning of all cultures and that no disturbing influence was introduced by soil conditions, it may be supposed that the periods characterized by exceptions to this generalization should also be characterized by some sort of corresponding peculiarities in the aerial environmental complexes. Now, a study of the charts for the exposed sta-

tions brings out the following fact: most of the 2-week periods for which both plant values are large and yet the index of stem elongation is greater than that of leaf-product increase, are characterized by low indices of sunshine intensity. This suggests that the plants of these cultures experienced an acceleration in their rates of stem elongation due to low light intensity, in short that they exhibited some of the effects of incipient etiolation. They seem to show a somewhat increased rate of stem elongation and a somewhat decreased rate of leaf expansion, as compared with plants receiving more radiation. This interpretation is not to be regarded as at all well established, but it is at least a suggestion of one way in which the external condition of light intensity and duration may be registered in such plants as were here employed.

*Trends of the 2-week plant values and their seasonal ranges for the several stations.* The following consideration of the seasonal marches of the 2-week plant values for the various stations will be limited in extent, since most of the facts and deductions that seem to be of importance in this connection can be better brought out later. Attention will here be called only to two characteristics of the plant graphs: (1) They begin with values of about 100 rise to high midsummer values and then fall to low values at the end of the season. (2) Differences in the magnitude of the midsummer maxima constitute the chief differences between the graphs for the various stations.

Oakland shows lower values for stem height and leaf-product than does any other station, due largely to the low temperatures prevailing at this station throughout the season. The data of this study indicate that the climate of Oakland, so far as it affects the plants, is very unlike that of any of the other stations employed. Both plant graphs for this station show the typical low values at the beginning and end of the season, however, with midsummer maxima of 124 for stem height and 89 for leaf-product. Chewsville shows typical graphs, the highest value reached by stem height being 143 while the leaf-product maximum is 139. The end of the season at this station is characterized by very low leaf-product values. The graphs for Monrovia are also typical, with low values of both indices for the period beginning May 18 and low values at the end of the season, after midsummer maxima of 121 (stem height) and 138 (leaf-product). An explanation of the low values shown for the Monrovia periods beginning September 7 and October 8 may lie in the fact that a minimum temperature only several degrees above freezing was reached during each of these periods. For both Chewsville and Monrovia the plant values are, for the most part, lower than 100, with relatively low midsummer maxima of 132 and 203, for stem height and leaf-product, respectively. The Baltimore plant graphs begin with high values and reach maxima of 183 and 233, for stem height and leaf-product respectively. For Darlington the main distinguishing features are the very high maxima of 228 for stem height and 295 for leaf-product, for the period

beginning July 10, and the relatively high values shown by the graphs for the beginning of the season. The midsummer maxima for Coleman are 157 (stem height) and 211 (leaf product). The plant graphs for Easton show relatively low values of the midsummer maxima, 146 being the highest value reached for stem height and 163 for leaf-product. Also, the plant values are low for the beginning of the season for this station. The midsummer maximum for stem height for Princess Anne is 197 and the corresponding maximum for leaf-product is 163.

The plant graphs, as may be seen from the above outline of their main features, fall into three groups: (1) *The Oakland graphs*, which show values of the leaf-product index below 100 for all periods and similar low values of the stem-height index for all periods except those beginning June 14 and August 14, while the maxima of these graphs are relatively low. (2) *The graphs for Chewsville, Monrovia, Princess Anne and Easton*, showing higher midsummer values of the plant growth-rates than do the Oakland graphs, the maxima being about one and one-half times the seasonal average. (3) *The graphs for College, Baltimore, Darlington and Coleman* which are distinguished by high relative values of their maxima. This classification serves to summarize such characteristics of the graphs as are of present interest.

*The 2-week climatic data for stations in the open (see figs. 2 and 3, red lines)*

The 2-week climatic data consist of the average daily relative values of the indices for temperature, evaporation, and light for each of a series of consecutive periods extending through practically the entire growing season, each period being about 14 days long. These values therefore furnish a continuous record of the growing season at each station. The 4-week periods, however, overlap, each one including the last two weeks of the preceding and the first two weeks of the following 4-week period, so that the climatic averages based on the 4-week data form a smoother curve than do the 2-week values in every case, small variations in the conditions being to a great extent obscured by averaging the overlapping periods. This series of 2-week values therefore exhibits the march of the climatic conditions at each of the various stations in somewhat greater detail than do the corresponding series of 4-week values. The former will therefore be made the basis for a somewhat detailed and comparative discussion of the climatic conditions at the various stations, temperature receiving attention first and light and evaporation being afterwards considered together. In each case, the general characteristics (common to most or all of the stations) of the seasonal march of the condition considered will be brought out, after which attention will be given to peculiarities of the values for individual stations.

## The 2-week temperature data

The most obvious general characteristic of the physiological temperature index is that its value is high for mid-summer and low for the beginning and end of the season, for all stations. Graphs of similar form are obtained when daily means and remainder summations are correspondingly plotted, but the midsummer rise is much more pronounced in the graph of physiological index values (here employed) than in either of the others. The second general characteristic of all the graphs of the physiological index of temperature is that each graph possesses two maxima, both of which have about the same magnitude. The first occurs for the last two weeks of July and the second for the last two weeks of August, this statement being true for all the stations considered except Oakland, for which station they both occur relatively early in the season, in the last two weeks in June and July respectively. A third feature which is common to most (though not all) of these graphs is that the upward slope is more gradual before the occurrence of the high midsummer maxima than is the downward slope after their occurrence. A generalized temperature-efficiency graph, representing averages of the corresponding values for all of the stations, is not symmetrical about the ordinate for its highest midsummer value; it slopes upward less rapidly than downward. A fourth general characteristic of these graphs lies in the fact that the final low index values of the frostless season are not very different for the various stations. The following consideration of the graphs for some of the individual stations will serve to bring out the points mentioned above and will give opportunity to note exceptions to the general statements just made.

With regard to the forms and other characteristics of the 2-week temperature-efficiency graphs, the nine stations studied may be placed in five groups: (1) Chewsville and Monrovia, (2) Baltimore, Darlington and Coleman, (3) Easton and Princess Anne, (4) College, and (5) Oakland. These five groups are discussed in order below. It will be noted that groups 1, 2 and 3 are composed of stations that are located near each other, and this probably accounts for the grouping.

*Chewsville and Monrovia.* The graph of physiological temperature indices for Chewsville shows all the characteristics mentioned as general throughout the series of stations. It rises gradually during the first three periods, (period beginning May 19 to period beginning June 16), then drops slightly during the fourth period (beginning June 30) after which it rises for the period beginning July 14 to a primary maximum of 149. The value for the 6th period (beginning July 28) is relatively low (112), after which a secondary maximum (145) occurs for the period beginning August 11. The index value in question then decreases rapidly during the next two periods attaining a magnitude of 48 for the 9th period (beginning September 8) and remaining low until the end of the frostless season. Monrovia has the same sort of graph as Chew-

cille, the maxima coming in the periods beginning July 13 and August 10. The minimum relative value of the temperature index is 53 for the period beginning September 21.

*Baltimore, Darlington and Coleman.* For Baltimore, the physiological temperature values increase gradually to a primary maximum of 162 for the period beginning July 29. The secondary maximum occurs in the period beginning August 6, after which there is a relatively rapid decline of the index values, to 62 for the period beginning September 3. The Darlington graph has its first maximum in the first two weeks of July and its second in the 2-week period beginning August 7, and then falls off rapidly to a minimum of 46 for the first period in September. The graph for Coleman shows a gradual rise, two maxima for the periods beginning July 8 and August 5, and a rapid fall. The temperature record is incomplete at this station and the low values for the end of the season are not available.

*Easton and Princess Anne.* For Easton there is a gradual rise to a maximum of 154, for the first period in July, the second maximum coming in the period beginning August 17. The curve then falls to a minimum of 48, for the last period of the season. The Princess Anne curve shows the two typical maxima in the periods beginning July 9 and August 18, with a minimum of 43 for the last period of the season.

*College.* The College graph of physiological indices is unusual in showing a marked rise for the period beginning June 19, thus giving the graph three maxima (129, 148 and 143, for the periods beginning June 19, July 17 and August 14, respectively). The graph descends rapidly to a value of 50, for the period beginning September 25.

*Oakland.* The temperature-index values for Oakland are all relatively low, being always considerably less than the seasonal average for all periods and stations. This graph shows two maxima, one for the latter half of June and the other for the latter half of July. Each of these maxima occurs about a month earlier than do the corresponding ones for the other stations here studied. The Oakland graph is also unlike those for the other stations in that its downward slope is more gradual. Its final relative value is 43, for the 2-week period beginning September 12, which was the last full period for this station before the occurrence of a killing frost. The most outstanding characteristics of the Oakland season, in respect to this temperature-efficiency graph, as compared with the seasons at the other stations, are: (1) general low values of the physiological temperature-index. (2) short duration, owing to the occurrence of late spring and early fall frosts, and (3) early occurrence of the maxima. These marked differences between the Oakland graph and those for the other stations here dealt with are no doubt largely due to the relatively high altitude of Oakland as compared with the others as has been mentioned by McLean in his comparative study of the Easton and Oakland seasons based on these same data.

*The generalized graph.* Leaving the graph for Oakland out of account, those for the other stations may be described as a single generalized graph, in the following general terms. Beginning with a relative index-value of about 80 (for the first part of May) the graph rises to a maximum (about 150) for the first part of July, falls slightly and rises again to a second maximum of about the same value as the first, for the first part of August, and finally falls to a minimum value of about 50 for the last period of the frostless season. That the initial values are not lower is no doubt due to the fact that the cultures were not started until somewhat after the beginning of the frostless season. (See McLean's paper, already cited.) This generalization of the temperature values for the various stations is not, of course, to be considered otherwise than as a statement of what occurred in the particular season during which this investigation was carried out.

#### Light and the evaporating power of the air, 2-week data

The 2-week graphs of the index values for light and atmospheric evaporating power will be treated together since the seasonal marches of these two climatic conditions generally exhibit the same main characteristics. Three points may be noted in regard to them. (1) Both graphs have, in general, a downward slope from the beginning to the end of the season. (2) In the majority of cases they agree with each other in direction of slope, from period to period, throughout the season. (3) They agree in having a primary maximum with a very high value, for an early period of the season and one or more secondary maxima with lower values, for periods that occur later. The secondary maxima of the graphs for light and evaporation sometimes (but not always) coincide, as to time of occurrence, with a corresponding maximum of the graph for temperature efficiency. The following consideration of the individual station graphs for the two conditions may serve to bring out these points.

For Oakland, the primary maximum in the graph of atmospheric evaporating power (153) occurs in the first period (beginning May 23). The value of the evaporation index then decreases steadily to a relative magnitude of 79, for the first two weeks in July, after which it increases to (104), which corresponds in time of occurrence (period beginning July 6) to the secondary maximum of the graph of temperature efficiency for this station. After passing through this high value the evaporation graph descends again, to the low values 57 and 69 for the last two periods (beginning August 27 and September 12). The sunshine-intensity index for Oakland varies from an initial value of 122 to a final value of 81, with maxima for the periods beginning July 16 and August 14. Inspection of these two graphs for Oakland shows that the direction of slope is the same, from period to period, for the greater part of the season.

For Chewsville, the two graphs agree in direction of slope throughout the entire season, except between the periods beginning August 25 and September 8. Both are approximately parallel to the temperature-efficiency graph for this station, from the period beginning July 14 to the period beginning August 25 and both have a downward slope, in general, from the beginning to the end of the season. Moreover, they agree in direction of slope from the period beginning June 15 to that beginning October 8.

For College, the evaporation maximum for the period beginning July 27 corresponds to a secondary minimum in temperature efficiency. The graph of the evaporating power of the air for College has a primary maximum for the second period (beginning May 28) and a well-marked secondary maximum for the period beginning July 22. No sunshine data are available for this station.

For Baltimore, the two graphs in question agree in direction of slope up to the period beginning July 9 after which evaporation passes through a secondary maximum which corresponds, in a very rough way, to the double maximum of temperature efficiency.

The Darlington light and evaporation graphs show the general characteristics mentioned at the beginning of this discussion, for the greater part of the season. The atmometric values for this station are relatively very low, all but two of them being less than the seasonal average for all periods and stations.

For Coleman, the sunshine record is incomplete, but the two graphs generally agree in direction of slope, so far as comparison is possible, excepting between the periods beginning July 17 and July 31.

For Easton and Princess Anne, the graphs are typical. For the latter station, evaporation data are lacking for the periods beginning June 8 and June 23.

The comparatively close agreement between the graphs for sunshine and evaporation, for all the stations employed in this study, together with the fact that evaporation exhibits no well-defined relation to temperature efficiency, appears to indicate that the rate at which water evaporated from the white cylindrical cups employed as atmometers in this investigation was determined to a considerable extent by the amount of radiant energy absorbed by the cups, and that air temperature played a secondary part in the determination of this rate. The fact that the physiological temperature index is here used for expressing temperature values does not militate against this conclusion, since, as has been previously stated other methods of expressing the temperature values give graphs which slope for the most part, in the same direction as does the graph of physiological temperature indices. A large effect of sunshine on evaporation, the sunshine intensity being measured by a black-bulb sunshine recorder, has been found by Briggs and Shantz.<sup>15</sup>

<sup>15</sup> Briggs, L. J., and Shantz, H. L. Hourly transpiration rate on clear days as determined by cyclic environmental factors. *Jour. Agric. Res.* 5: 583-650. 1916.

These authors were able to calculate approximately the amount of evaporation from a shallow blackened tank using a formula which involved sunshine intensity and the saturation deficit of the air, sunshine intensity having a preponderating influence. They also state that while the cups and the tank respond in different ways to the daily cycle of changes in the evaporating power of the air, a certain average ratio exists between the evaporation from the tank and that from the cups. It is therefore to be expected from their work that the rate of evaporation from Livingston porous cups is largely influenced by sunshine intensity, and that air temperature exerts a secondary influence on evaporation as measured by these instruments. It must be remembered, also, that the evaporation measurements of this study were made in the plant enclosures, while air temperature was measured by thermometers located in a shelter about 1.5 meters (5 feet) above the ground and often 4 or 5 meters (15 feet) from the plant enclosures. This may account in some measure for the apparent absence of any marked effect of air temperature on the evaporating power of the air as measured by porous-cup atmometers. As Livingston has remarked, the porous cups are exposed in somewhat the same way as are plant leaves, and the foliage of McLean's plants was freely exposed to sunshine, as were his atmometers also. Air temperature is always obtained from shaded instruments.

#### Variability of temperature and evaporation values

It may be noted that the temperature-efficiency values for the stations here considered, exclusive of Oakland, are much more nearly alike for any given 2-week period than are the sunshine and evaporation values. The values of these three climatic indices for the first 2 weeks of June and for the first 2 weeks of August, for the eight stations, are given in table IX. Since the dates of observation were not the same for all stations, these values have been approximated from the graphs, but they may be considered as sufficiently accurate to illustrate the manner in which the data at hand support the conclusion just stated.

If the highest value given for each of the three indices and for each of the two periods be divided by its lowest value, the ratios presented in the next to the last line of the table are obtained. Each ratio represents the magnitude of the range of variation of the climatic index that it represents, for the eight stations in question. The average value for these periods is given in the last line. It thus appears that the variation of the temperature-efficiency index due to difference in location of the stations is markedly less than is the corresponding variation in the index of sunshine or that of evaporation. This relation holds generally throughout the season. In short, the temperature-efficiency values exhibit a smaller degree of geographical or local variation than is exhibited by the index for sunshine or for the evaporating power of the air.

TABLE IX

*Values of the three climatic indices for the first 2 weeks in June and the first 2 weeks in August, with ratio of highest to lowest value for each index, for all stations excepting Oakland.*

STATION	EVAPORATION		SUNSHINE		TEMPERATURE EFFICIENCY (PHYSIOLOGICAL INDEX)	
	1st 2 weeks of June	1st 2 weeks of August	1st 2 weeks of June	1st 2 weeks of August	1st 2 weeks of June	1st 2 weeks of August
Chewsville.....	115	90	130	95	105	120
Monrovia.....	146	115	122	103	110	125
College.....	156	147	—	—	103	133
Baltimore.....	109	110	92	77	102	152
Darlington.....	115	78	115	112	98	125
Coleman.....	145	135	145	120	115	152
Easton.....	132	135	165	115	112	140
Princess Anne.....	125	95	110	75	102	135
Ratio of highest to lowest value in above series.....	1.5	1.9	1.8	1.6	1.2	1.3
Average for the 2 periods.....	1.70		1.70		1.25	

*Correlation of the 2-week plant and the climatic values*

During the course of this study a number of attempts were made to correlate the climatic measurements with those representing the growth rates of the plants, but these were unsuccessful and no scheme applicable in a quantitative way to this problem has yet been formulated. For example, one of the simpler correlation schemes to be tried was based on the assumption that the growth of the plants was directly proportional to the index values for temperature and light and inversely proportional to those for evaporation. Stated as an equation, this assumption takes the form:

$$G = \frac{K T L}{E},$$

in which  $G$  represents the plant growth rate and  $T$ ,  $L$ , and  $E$  represent the indices of temperature, light and evaporation, respectively, while  $K$  is a constant of proportionality. Values were obtained for the right-hand member of this equation for the successive 2-week periods for all stations and these values were compared with the corresponding growth-rate indices derived from the plant measurements. No close correspondence was generally to be detected. The equation is given as an illustration of the kind of methods by which the discovery of correlations between the plants and their climatic environment was attempted. Many combinations of the three climatic

conditions were made and compared with the plant growth rates but, as noted above, without satisfactory results.

It seems probable that the difficulty experienced by every student who has thus far attempted this sort of correlation may arise partly from the fact that the environmental conditions have not been measured in the right way, and partly from the use of inadequate methods for the integration of the quantitative data that are obtained. It is hardly to be expected that either of the growth criteria here used should be as simple a function of the climatic conditions as the formula given above might suggest. Just as soon as facilities become available for actual experiments in this field,—experiments in which all the influential conditions may be controlled and analytically understood,—the problem here brought forward prematurely may be seriously attacked. Until such experiments may be begun, all discussion regarding the relations between plant growth rates and environmental conditions must remain vague and unsatisfactory.

There is no doubt that the *distribution of high and low values* of any one of the climatic conditions, during the growth period of the plants, is an important factor in determining the degree of their development. To take an extreme case as an example, a few days with a very low sunshine intensity would have no direct influence on plants not yet above ground, but such an occurrence would exert a very marked influence on plants with a considerable leaf area. Obviously, two periods showing similar *average* values of any climatic condition may have a widely differing distribution of high and low values of this condition. In the present study, while the distribution of high and low temperature and light values is known, the corresponding stages of the development of the plants are not, and it is thus practically impossible to take account of this distribution factor. The difficulty of correlating growth rates and climatic conditions is further increased by the fact that, in measuring dry weight, stem height, etc., we are not measuring single processes in the plants, but rather the combined effects of a number of processes taken together.

It is of interest to call attention at this point to certain features of the growth of the soy-bean plants of this study whose causes can only be surmised. These features may be of significance, however, since they show a departure from what may be termed the "normal" for plant behavior. In the first place, although all of the temperature graphs show two maxima, the plants, except in the case of Oakland, failed to respond to the second temperature maximum by a correspondingly high rate of growth. For Chewsville, as an example, in the period beginning August 11, we have a low value for the leaf-product with a high temperature index and the other conditions at about the seasonal average. For Monrovia in the period beginning August 10, with a high temperature value and with sunshine intensity at about the seasonal average, the plants show a relatively low value of the leaf-product.

This may be contrasted with the period beginning June 15 for this same station, which, with a leaf-product about the same as that of the first-mentioned period, seems to show less favorable growing conditions—namely, a much lower relative temperature index, a very high evaporation rate and a sunshine value only a little higher than the corresponding value for the period beginning August 10. For College, the periods beginning July 17, July 31 and August 14, with about the same values for temperature and evaporation, show magnitudes of 152, 204 and 150 respectively for the leaf-product. This variation may possibly be related to differences in the value of sunshine intensity for these periods, but sunshine data are lacking for this station. For Baltimore, the periods beginning July 23, August 6 and August 20 show large differences in the leaf-product with comparatively slight differences in the climatic conditions. Evaporation was slightly less rapid for the period beginning July 23 than for the period beginning August 8, and considerably less for the period beginning August 20, but this seems to have occurred without the expected effect on the plants. For Coleman the plant graph slopes upward to a value of over 200 for the period beginning July 8, while for the period beginning August 5, which has climatic conditions apparently as favorable, the relative value of the leaf-product is only 138. For Easton the leaf-product is lower than would be expected for the period beginning August 17, and for Princess Anne the plant values for the period beginning August 8 are much lower than for the period beginning July 7, which had approximately the same climatic conditions as the first-mentioned period.

A second feature of the plant graphs, and one that cannot be correlated with the climatic data, is that the rate of stem elongation reaches its highest value for the season before the occurrence of the maximum leaf-product, for all stations except Darlington and Coleman. For Darlington, the highest value for stem height and leaf-product both occur for the period beginning July 10 and for Coleman the maximum value for stem height occurs for the period beginning July 22, while the leaf-product reaches its highest value for the season at this station in the preceding period. For the remaining stations, the highest value for stem height occurs two weeks or a month earlier than does the highest value for leaf-product.

#### THE 4-WEEK VALUES

The 4-week plant and climatic data derived from the exposed stations are presented in the tables and graphs already explained, and the following consideration of these values will refer to the graphs, as in the case of the 2-week values.

*The 4-week plant data for stations in the open (see fig. 4, black lines)*

For the 4-week data, the rate of stem elongation may be compared with the rate of leaf expansion as determined from actual measurements of *leaf area*. This comparison shows the same general relations as appeared to exist between stem height and leaf-product for the 2-week growth periods. Owing to the fact that the 4-week plants were grown for a longer time, however, the 4-week data show fewer cases with the rate of stem elongation greater than the rate of leaf expansion. In most cases the rate of stem elongation is considerably smaller than the rate of leaf expansion. This illustrates the tendency of the soy-beans to show a low rate of height growth relative to the rate of leaf expansion when both rates are large.

For Oakland, the stem-height graph is above the leaf area graph for the first three periods of the season and below it for the other five periods. For Chewsville the three plant graphs follow each other very closely and the differences in their relative positions are probably due, for the most part, to individual variations in the plants of the separate cultures. The Monrovia graphs also support the assumption that stem height shows a well-defined tendency to remain below leaf area during the first part of the season. For College, the stem-height graph is below that for leaf area for the entire season, except for the two periods beginning June 19 and September 25. The Baltimore graphs show stem-height values higher than the corresponding leaf-area values for the periods beginning May 14, May 29, June 10 and August 20, due possibly to low light intensities. The Darlington cultures show very high values of both growth rates, with stem height below leaf area for the entire season. For Coleman, the stem-height graph remains below the leaf-area graph for all the periods except the last, in which case it rises very slightly above the leaf-area graph. For Easton, the two growth rates are about alike, showing nearly the same relative values for each culture period. For Princess Anne, the stem-height and leaf-area graphs show a departure from the usual behavior during the first three periods of the season. For these periods, the leaf-area values are relatively large and those for stem height are relatively low, for some reason not apparent from the climatic conditions.

A very striking relation is shown between the 4-week values for leaf area and for dry weight. For most of the cultures these two kinds of growth rates have practically the *same relative numerical values* for any given period. The Oakland graphs show this for all periods except the one beginning June 5, for which dry weight is markedly larger than leaf area. For Chewsville, the general relation just mentioned shows very well throughout the season. For Monrovia, the leaf-area value shows a rather large deviation from the dry-weight value for the periods beginning June 16, June 30 and Aug. 25, but otherwise the two growth rates correspond in their relative values during the

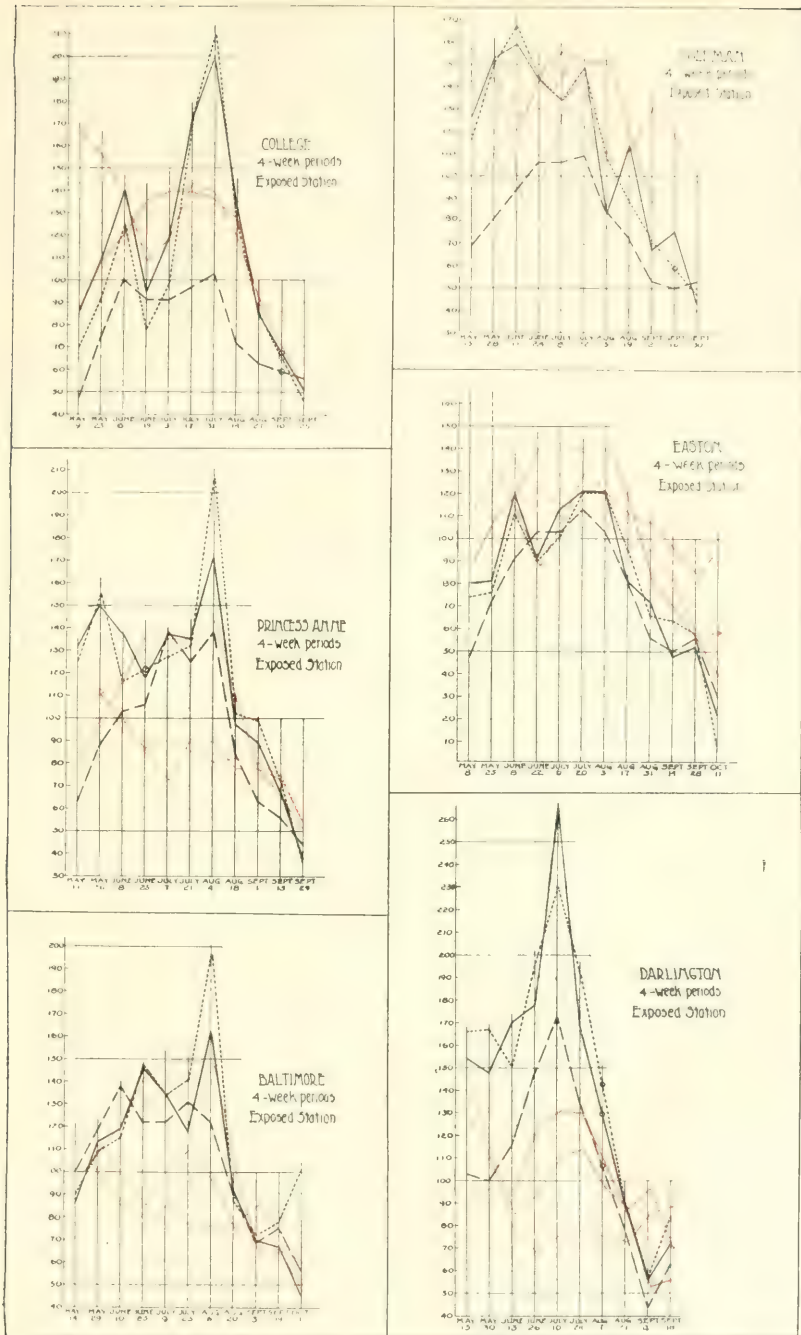


FIG. 4. Graphs of 4-week data for exposed stations as named (continued).  
(Lines as in 4-week graphs of fig. 3.)

entire season. The College graphs show close agreement, with dry weight above leaf area during the first part of the season. For Baltimore, the relative leaf-area value differs considerably from the value for relative dry weight for the periods beginning August 6 and October 1, but the remaining periods show close agreement. The two Darlington graphs show close agreement for all periods. For Coleman, dry weight and leaf area agree well for all periods, except those beginning August 5 and August 19. For Easton, no large differences between these two rates occur for any of the cultures. For Princess Anne, the period beginning August 4 is the only one showing a difference of considerable magnitude between the relative leaf-area value and the dry-weight value.

This property or characteristic of soy-bean renders possible the use of the leaf area of the plant as an index of the dry weight of the tops, and appears to render soy-bean particularly promising as a standard plant for climatic investigations, as has been pointed out in a previous paper,<sup>19</sup> from which the following paragraph is taken.

"If the method proposed by Livingston and McLean (1916), of employing the growth rates of standard plants as indices for the comparison of different climates as these influence plant growth in general, is to be of value, it is of course necessary that suitable plant characteristics be chosen for measurement in determining the growth rates, and it is desirable that the measurements be such as may be made from time to time without injury to the plants. The most generally accepted criterion of plant growth, dry weight of tops, can be obtained but once for any individual plant, since the plant is destroyed during the determination. Also, the accurate determination of leaf area is very difficult unless the plants are destroyed. On the other hand, as McLean has emphasized, leaf dimensions may be obtained repeatedly during the development of the plant, without serious danger of inflicting injury. It may therefore be of considerable importance if leaf area, and even dry weight can be satisfactorily estimated for soy-bean by the employment of the leaf-product as an index."

Dry weight and actual leaf area were both determined only for the 4-week periods, the plants being then destroyed, but the lengths and breadths of all leaflets were obtained for both the 2-week and the 4-week periods. Consequently, to study the correlation between total leaf area and total leaf-product per plant, only the 4-week data are available and these are the ones here considered.

Since soy-bean leaflets are approximately elliptical in form and since the area of an ellipse is proportional to the product of its axes, the leaflet-product (length times breadth) of any leaflet should be nearly proportional to the area of that leaflet. Whether this relation may hold during the growth of

<sup>19</sup> Hildebrandt, F. M. Leaf product as an index of growth in soy-bean. Johns Hopkins Univ. Circ., March, 1917. P. 202-205.

the leaflet under different sets of climatic conditions depends upon how nearly the elliptical form is retained. The sum of the individual leaflet-products of any plant, which is the total leaf-product for that plant, should be approximately proportional to the total leaf area of the plant, if the relation given above holds. In the discussion that follows it will be shown that such an approximate proportionality does exist in the case of the 4-week soy-bean plants.

In order to find out whether the actual areas of the leaves in these cultures were proportional to the corresponding leaf-products, the ratio of the two quantities was worked out for a number of the stations. It was found that the leaf-product divided by the leaf area gives a number that varies only slightly from the value 1.28. In other words, if we measure the two diameters of the leaflets of a 4-week soy-bean plant, multiply these two numbers for each leaflet, and add the products, a number is obtained which, when divided by 1.28, closely approximates the actual leaf area of that plant. Instead of using the sum of the products of length and breadth as an index of the area per plant we may use the sum of the squares of the lengths of the leaflets or the sum of the squares of the breadths of the leaflets. The numbers thus secured do not, however, bear as nearly constant a ratio to the actual leaf area as does the total leaf-product, and hence neither is as satisfactory an index of the area as is the leaf-product itself.

One of the most interesting properties of the 4-week soy bean plant is that the dry weight of stem and leaves is approximately proportional to the total leaf area. Having, therefore, a means by which the leaf area may be conveniently estimated, it is possible to calculate the dry weight of the plant approximately, by multiplying the leaf-area by the proper constant. The proportionality between the weight of the plant and its leaf area is not quite so constant as that between leaf area and leaf-product, but in the great majority of cases the variation in the ratio of dry weight to leaf area, from a constant value, is less than 10 per cent. The relations given hold over a very wide range of climatic conditions and for plants varying in height from 2 or 3 cm. to 18 or 20 cm. Since none of the plants in these experiments were grown to maturity, it is impossible to say whether this relation holds up to that time.

From the foregoing facts it may be concluded that the dry weight and leaf area of soy-beans 4 weeks old from the seed can be determined approximately from their leaflet dimensions. Soy-bean should therefore be very suitable for use as a standard plant for the measurement of climate in the manner suggested by Livingston and McLean, since the rate of its growth can be approximately determined from easily obtained leaf measurements. Also, the properties of soy-bean given above should make it a useful plant for any piece of physiological research in which it is desired to know approximately the dry weight of the plant used, at various stages of its development.

*The 4-week climatic data for stations in the open (see fig. 4, red lines)*

It will be remembered that the cultures were started every two weeks and that each grew for a period of four weeks. The 4-week periods thus overlap, and attention has been called to the fact that averages of the climatic factors for these over-lapping periods form a smoother graph than averages for the 2-week periods. The 4-week graphs, therefore, show the general seasonal march of the index values for various stations better than do the 2-week ones, while the latter show the details of the seasonal march better than the former. This fact will be brought out by a brief reference to the graphs at this point.

The values of the physiological temperature indices for the 4-week periods show the seasonal marches of this condition for the various stations, from low values in May to high midsummer values, and then to low values again in the last part of the season. The graphs for all of the stations except Oakland show a steeper slope after the midsummer maximum has been passed than for the periods during which the temperature was rising to this maximum. The two maxima that were present in most of the 2-week graphs are eliminated in the 4-week averages and the graphs of temperature values show instead a period of about 6 weeks during which this condition remains approximately constant.

The 4-week evaporation and light data show the general characteristics of the seasonal marches of these conditions previously noted as exhibited by the 2-week data. It will be seen, in the first place, that both graphs exhibit a downward slope from the beginning to the end of the season; and, in the second place, that both graphs show, in addition to their high primary maximum in the early part of the season, one or more secondary maxima later. In some cases the secondary maxima of the evaporation graphs coincide, as to time of occurrence, with temperature maxima. Both of these general characteristics shown in the 4-week graphs of evaporation and light are shown by the 2-week graphs but since small variations are eliminated by averaging the over-lapping periods, there are fewer secondary maxima in the 4-week graphs. In the case of evaporation, there is usually one secondary maximum occurring in or near the 4-week period including the last 2 weeks of July and the first 2 weeks of August. In the case of all stations this is one of the three 4-week periods showing high temperature values. The 4-week climatic graphs need not be discussed further here. The method by which the 4-week data were derived from the 2-week data amounts to the same thing as smoothing the 2-week graphs and only the more pronounced characteristics of the graphs remain after averaging. Interest in the 4-week climatic data thus lies mainly in their relation to the plant growth rates.

## RESULTS FOR THE THREE COVERED STATIONS

## INTRODUCTORY

All of the data discussed up to this point were obtained for the open, with no covering other than a screen of wire netting of large mesh, to protect the plants from injury. At three of the stations, Oakland, Baltimore and Easton, as has been noted, a series of cultures was also grown under glazed cold-frame sash, supported three feet above the ground, these cultures being designated as the Oakland, Baltimore and Easton *covered* stations. The behavior of the plants grown under glass was very different from the behavior of those grown in the open, and the results for the covered stations will be considered in this section.

The covered cultures were placed near the exposed cultures at each of the three places mentioned, so that the climatic conditions for the two would be practically the same, except as modified by the glass.

## THE PLANT DATA, COVERED STATIONS

(See *figs. 5 and 6, black lines*)

The effect of the glass cover was shown by the plants in two ways: (1) growth was always greater for the covered stations than for the exposed, and (2) the plants of the covered stations showed a marked difference in manner of growth from the plants of the exposed stations. The greater growth of the covered plants was shown in some cases by one, in some cases by two, or even by all three of the growth measurements taken. Not only did the plants show greater growth, but the maxima in the graphs of the various growth measurements for the covered plants do not usually occur at the same times as do the maxima in the corresponding graphs for the plants grown in the open. The principal effect of the covering on the way in which the plants grew is shown by a disturbance of the relation between dry weight and leaf area. In previous discussion of this relation for the exposed plants it was noted that the relative dry-weight and leaf-area values are approximately the same for the 4-week plants. In the case of the covered stations, on the other hand, every culture shows relative leaf area as higher (usually very much higher) than relative dry weight. Stem height for the covered cultures usually shows high values as compared to the corresponding exposed cultures. The tendency noted in previous discussion for this growth rate to fall off relatively, as the plants become larger, seems to be only slightly in evidence here. The following consideration of the covered cultures in detail will bring out these features. It should be noted that the culture periods for the covered stations each agree in length, to within a day or two, with those for the corresponding exposed stations. Such slight differences as exist in

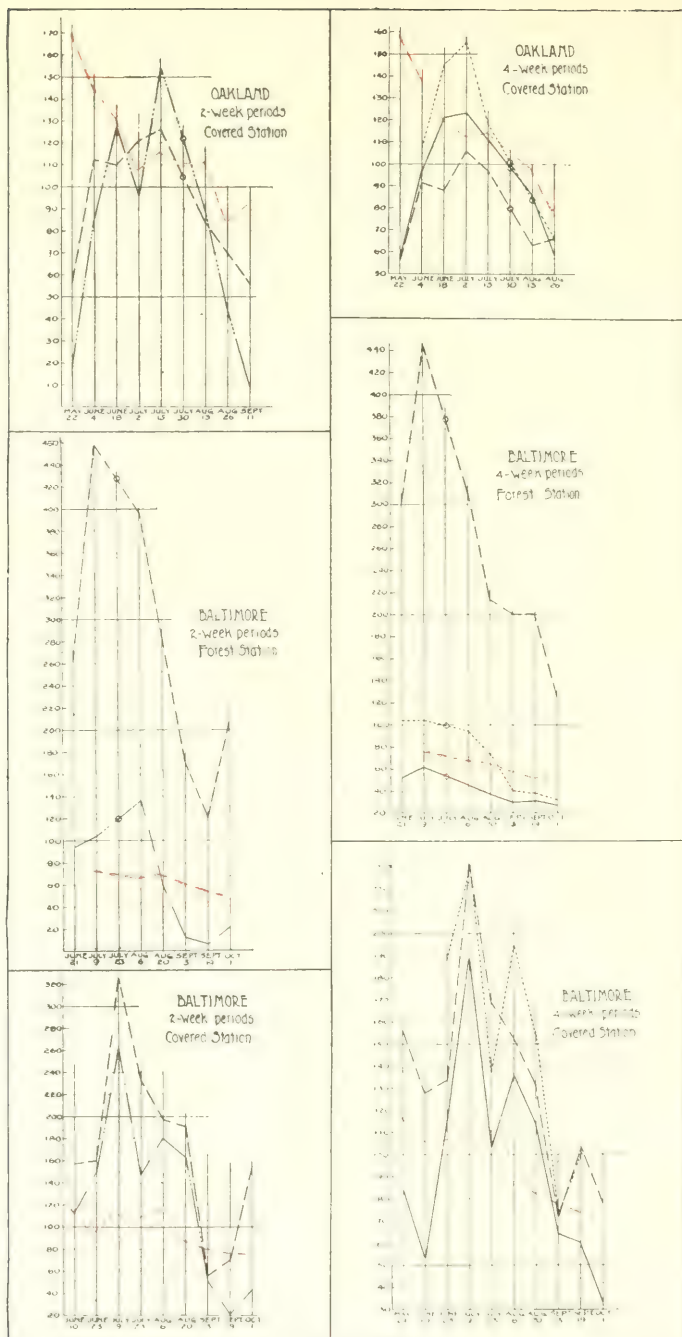


FIG. 5. Graphs of 2-week and 4-week data for covered and forest stations, as named.

Black, as in figs. 1 and 3.  
Red, Evaporation index.

the lengths of the culture periods do not in any degree account for the differences in the plant measurements nor interfere with the general comparisons here made. In comparing growth for the exposed and covered cultures, no attempt will be made to account in detail for the differences between the two sets of plants in terms of climatic conditions, since the climatic influences acting on the covered plants are not even so well known as in the case of the exposed stations, and it has already become clear that a really satisfactory interpretation of growth rates by means of such climatic measurements as are here employed is nearly hopeless at present. After the peculiarities of the covered plants have been pointed out, however, some suggestions as to the probable causes of these peculiarities will be brought forward.

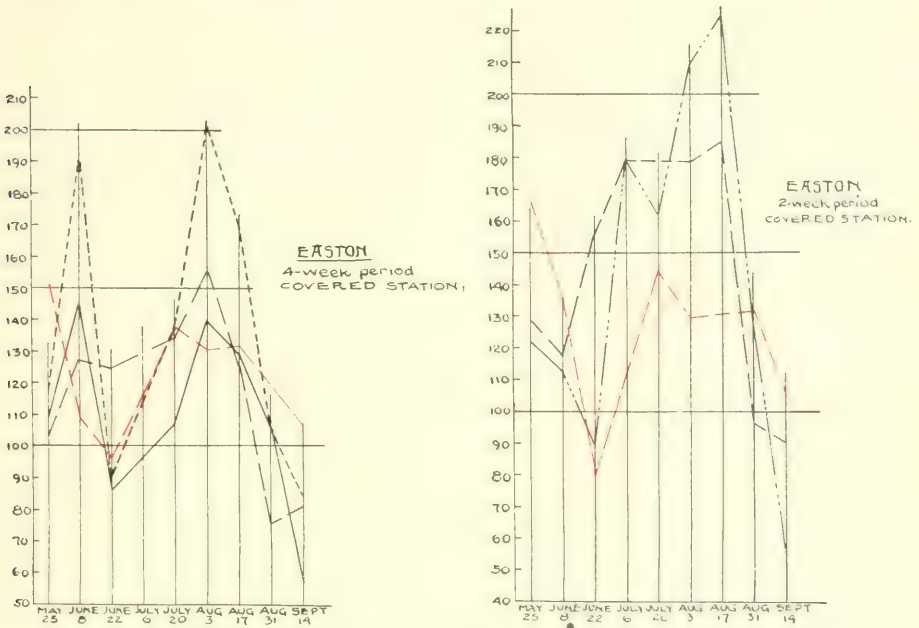


FIG. 6. Graphs of 2-week and 4-week data for Easton covered station.  
(Lines as in fig. 5.)

*The Oakland covered station.*—The covered and exposed cultures for Oakland differ less than do the corresponding sets for Baltimore and Easton, but they show the general features outlined above. The plants of the 2-week covered cultures for Oakland exhibit a much higher value of the leaf-product than do the corresponding exposed cultures, for the periods beginning June 18, July 2, and July 15, and the stem-height value is greater for the covered station than for the exposed station, for the periods beginning June 4, July 2 and July 15. The highest value of leaf-product occurs for the period beginning July 15 for the covered, and in the period beginning July 16 for the

exposed 2-week plants. Each set of cultures show two seasonal maxima in the plant graphs, but these are much higher in the case of the covered plants than in the case of the exposed. In the 4-week graphs for the covered plants, leaf area is higher than dry weight for the whole season, while the exposed-station graph for leaf area is well below that for dry weight, from the period beginning May 23 to the period beginning July 16, inclusive. The maximum for all the growth measurements of the 4-week exposed plants occurs for the period beginning June 19, while the maximum for the covered station occurs for the period beginning July 2. Also, the graphs for the 4-week plants all exhibit higher values than do the graphs for the 2-week plants for most of the culture periods of the season. This is especially true of leaf area. Covering the plants with glass seems to have produced a relatively high rate of leaf expansion, in spite of the fact that the evaporation value is somewhat higher for the covered than for the exposed plants.

*The Baltimore covered station.*—The 2-week plant data for the covered station at Baltimore are plotted to a scale one-half as great as the scale used in plotting the exposed plant values, on account of the high values of stem height and leaf-product shown by the covered culture beginning July 9. The values of both leaf-product and stem height for this station are both uniformly above the corresponding values for the exposed station. Also, the tendency of the covered plants to elongate relatively more rapidly than did the exposed plants is shown by the stem-height values for Baltimore covered station for both the 2- and 4-week periods. It is interesting to note that the covered plants do not show specially high values of the plant growth rates for the period beginning August 6, as do the exposed plants. The 4-week graphs for the covered plants show very well the tendency of leaf area to reach values relatively higher than those for dry weight, the leaf-area graph being well above the dry-weight graph for the entire season.

*The Easton covered station.*—For Easton the covered plants, as compared with the exposed, show the general tendencies noted above. The 2-week growth rates of the covered plants, especially for stem height, are higher than the corresponding rates of the exposed plants. It will be observed that the maximum growth for the season, in both the covered and exposed 2-week cultures occurs for the period beginning August 3. The 4-week plants of the covered cultures show leaf area relatively higher than dry weight. The values for the culture period beginning June 22 are relatively low for the covered as well as for the exposed cultures.

#### THE CLIMATIC CONDITIONS, COVERED STATIONS

(See figs. 5 and 6, red lines)

Of the three climatic factors generally dealt with in this study, evaporation alone was measured for the covered stations, so that the climatic data are much less satisfactory in this case than in the case of the exposed stations.

It is safe to suppose that the climatic conditions under the glass differed from those for the corresponding exposed stations in certain definite ways, period by period. The rate of evaporation for the covered stations was considerably greater than for the exposed as will be seen by comparing the values given in the tables. We may be certain, also, that some of the incident light was absorbed by the glass and that the light intensity under the cover was thus less than the intensity of the light falling on the exposed plants. Also, we may be reasonably sure that the air temperature under the glass was somewhat higher than that outside, especially on quiet days when circulation of air was slight, and there was little tendency toward equalization of air temperatures. In considering the behavior of the covered cultures as related to climatic conditions, it may be mentioned that evaporation is known to have been more intense and light intensity lower for these than for the corresponding exposed stations and periods, while air temperature was probably higher for the covered than for the corresponding exposed stations.

The differences between the behavior of the plants under glass and that of the plants in the open seems to be primarily attributable to differences in light conditions for the two sets of cultures. The more rapid stem elongation occurring under glass is exactly what would be expected if the air temperature was higher and the light intensity was lower than in the case of the corresponding exposed cultures. The fact that leaf area is relatively high for the covered plants, as compared with their final dry weight, may possibly be related to a smaller amount of dry matter produced by photosynthesis per unit of leaf area in the covered cultures. Such a difference might be expected if the light energy available for photosynthesis were cut down by interposing between the plant and the light source a screen that absorbed a part of the light.

Whatever may be the true explanation of the behavior of these plants under glass (and the true explanation will surely be much more complicated than is here suggested), the facts indicate very clearly that the growth of the plants under glass was quite different from the corresponding growth in the open. This point must be important in physiological experiments conducted in greenhouses.

#### RESULTS FOR THE BALTIMORE FOREST STATION

(See *fig. 5*)

The Baltimore Forest Station was located about 150 yards from the exposed and covered stations at that place. Evaporation was the only climatic feature measured for this station. The sunshine intensity was of course very low, due to the shading and screening effect of the leaves of the trees above the experimental plants. Air temperature was also probably

considerably lower than that experienced by the exposed and covered plants. The modification of growth habit in the case of the forest plants is very striking, as can be seen by an inspection of the plant graph for this station. The soy-beans were short erect growers in the open, and were erect with long stems under the glass of the covered station, but were *runners* in the forest. This effect on stem growth, which obviously cannot be explained as an effect of temperature alone in the case of these cultures, is relatively very great, the highest 2-week value for stem elongation being over four and a half times as great as the seasonal average for all periods and stations, and the highest 4-week value was a little less than four and a half times the seasonal average. As compared with plants grown in the open, the 4-week forest plants also show the same reversal in the relative positions of the leaf-area and dry-weight graphs as was shown by the covered plants. The leaf-area graph is above the dry-weight graph for the entire season in the forest. These cultures are thus more like the covered ones than they are like the exposed ones. This may possibly be accounted for by supposing that the similarity in the behavior of the plants in the covered and forest stations at Baltimore was related to a corresponding similarity in the light conditions for these two sets of cultures, but the problem is doubtless very complex.

## THE PLANT DATA AS MEASURES OF THE CLIMATIC EFFICIENCY FOR GROWTH OF THE STANDARD PLANTS

### INTRODUCTORY

As has been stated, the investigation of which this study is a part was planned with the idea of obtaining some quantitative measures of the climatic complex for each of the various stations, in terms of plant activity. Since the soil used was the same, since its moisture content was kept high enough to support good growth at all times, for all stations and for all periods, and since seeds of the same lot were used in all cases, it is supposed that the differences in the growth rates for the various periods and stations must have been due to effective environmental differences other than those of soil conditions. On account of the auto-irrigation of the cultures, precipitation was practically without direct influence upon the cultures of the exposed and forest stations, and it was of course quite without direct influence upon the cultures of the covered stations. The influential environmental conditions that differed from station to station and from period to period in these tests were those usually considered as climatic, with the omission of precipitation. The plant data, as set forth in the tables and graphs, may therefore be regarded as approximate measures of the integrated non-precipitation conditions of the several climatic complexes under which the plants grew. These

measures of course refer specifically to this particular variety of soy-bean plant and to the particular set of soil conditions that was common to all cultures. With another soil, or with another kind of plant, the plant values would of course have been more or less different from those here recorded. It remains to be found out whether or not soy-bean is a suitable standard plant for use in this sort of climatic integration when the needs of agriculture, forestry and general ecology are primarily considered. From what has been said in the preceding sections it appears, however, that soy-bean is at least especially well suited to preliminary and pioneer studies like the present one.<sup>20</sup>

From this point of view, each of the graphs of the plant values (shown by the black lines in figures 2-6) may be regarded as a representation of the seasonal march of the non-precipitation portion of the climatic environment for the particular station in question, the graphs for the exposed stations representing the "natural" conditions, while those for the covered and forest stations refer to the more or less modified climates experienced by these cultures. Some of the more outstanding features of these plant graphs have been mentioned in the preceding sections of this paper, and other features will become evident from a careful study of the graphs themselves, or of the tables from whose data the graphs were constructed. Much more might be said in this connection than has been said, but the newness of the present point of view, together with the obvious complexity of the numerical results here presented, make it undesirable to attempt a careful study of these data at the present time. The tables and graphs of this paper render the numerical values available for future study, when this aspect of climatology and ecology shall have begun to attract more general and appreciative attention than it now enjoys.

It should be emphasized that the plants have automatically weighted and integrated all the fluctuating and differing conditions for the several culture or exposure periods, and that the final summation is given in terms of the amount of growth produced in 2 weeks or 4 weeks from the seed. Dividing this final summation by the number of days in the corresponding period gives the average plant producing power of the non-precipitation part of the climatic complex for the given period and station.

It has been noted that these plant values generally show a seasonal march for each station, the growth index being relatively low for periods near the beginning and end of the season, and relatively high for midsummer periods, and it has been suggested that temperature may be considered as the main controlling condition in the bringing about of these seasonal marches, various modifications being superimposed upon the temperature influence by other climatic conditions such as the intensity, duration and seasonal distribution of light, and the intensity and seasonal distribution of evaporation.

---

<sup>20</sup> A study somewhat similar to this one, using wheat, pea and brome-grass as standard plants, was carried out by Sampson assisted by the author. See: Sampson, A. W. Climate and plant growth in certain vegetative associations. U. S. Dept. Agric. Bull. 700. 72 p., 37 fig. Govt. Printing Office: Washington, 1913.

## SEASONAL AVERAGES OF MEAN DAILY INTENSITY VALUES FOR THE SEVERAL STATIONS

Aside from the characteristics of the seasonal marches of the climatic conditions in question (which are best seen in the seasonal graphs themselves, figs. 2-6), it is of interest to average all the corresponding plant-index values for the season for each station, thus obtaining a seasonal average or mean daily plant-producing power, as a single index for each growth criterion for each station. This has been done for all the stations, for the 2-week and for the 4-week periods and for each growth criterion, and the resulting seasonal

TABLE X

*Relative seasonal daily means for the several stations, by each of the five growth criteria. The letter H denotes high values; M, intermediate values; and L, low values. (The covered station and the forest station are included for completeness.)*

STATION NAME	NUMBER OF DAYS	2-WEEK STEM HEIGHT	2-WEEK LEAF PRODUCT	4-WEEK STEM HEIGHT	4-WEEK LEAF AREA	4-WEEK DRY WEIGHT
Oakland. ....	125	L86	L77	L71	L71	L79
Chewsville.....	154	L87	L84	L75	L74	L78
Monrovia.....	154	L79	L80	L66	L71	L79
College.....	154	M95	M101	M80	M110	M119
Baltimore.....	153	H125	H119	H104	M115	M105
Darlington.....	154	H113	H118	H106	H148	H144
Coleman.....	168	M96	M107	M80	M117	M113
Easton.....	171	M95	M105	M75	L82	L83
Princess Anne.....	169	M106	M96	M92	M117	M116
Oakland, covered.....	—	M92	L78	M81	M118	M93
Baltimore, covered.....	—	H172	H125	H136	H154	M95
Easton, covered.....	—	H145	H142	H116	H137	M109
Baltimore, forest.....	—	HH <sup>a</sup> 271	LL <sup>a</sup> 62	HH <sup>a</sup> 255	L69	LL <sup>a</sup> 41

<sup>a</sup> The doubling of a letter indicates an extreme condition; HH means *very* high, etc.

means are shown in the last column of each of the data tables (tables I-VIII), where the corresponding seasonal averages for the climatic data are also given. It is to be remembered that the values are all relative, each one being stated in terms of the corresponding average for all stations and all periods, this unit being considered as 100.

The seasonal averages for the various exposed stations are brought together in table X and are shown graphically in figures 7 and 8, the former figure dealing with the 2-week and the latter with the 4-week plant data. The abscissas of these graphs are not quantitative; the vertical lines are equally spaced and each one represents one of the exposed stations. The stations are arranged in the order of their geographical locations, as far as this is possible in a linear series. The ordinates of these graphs represent the seasonal means.

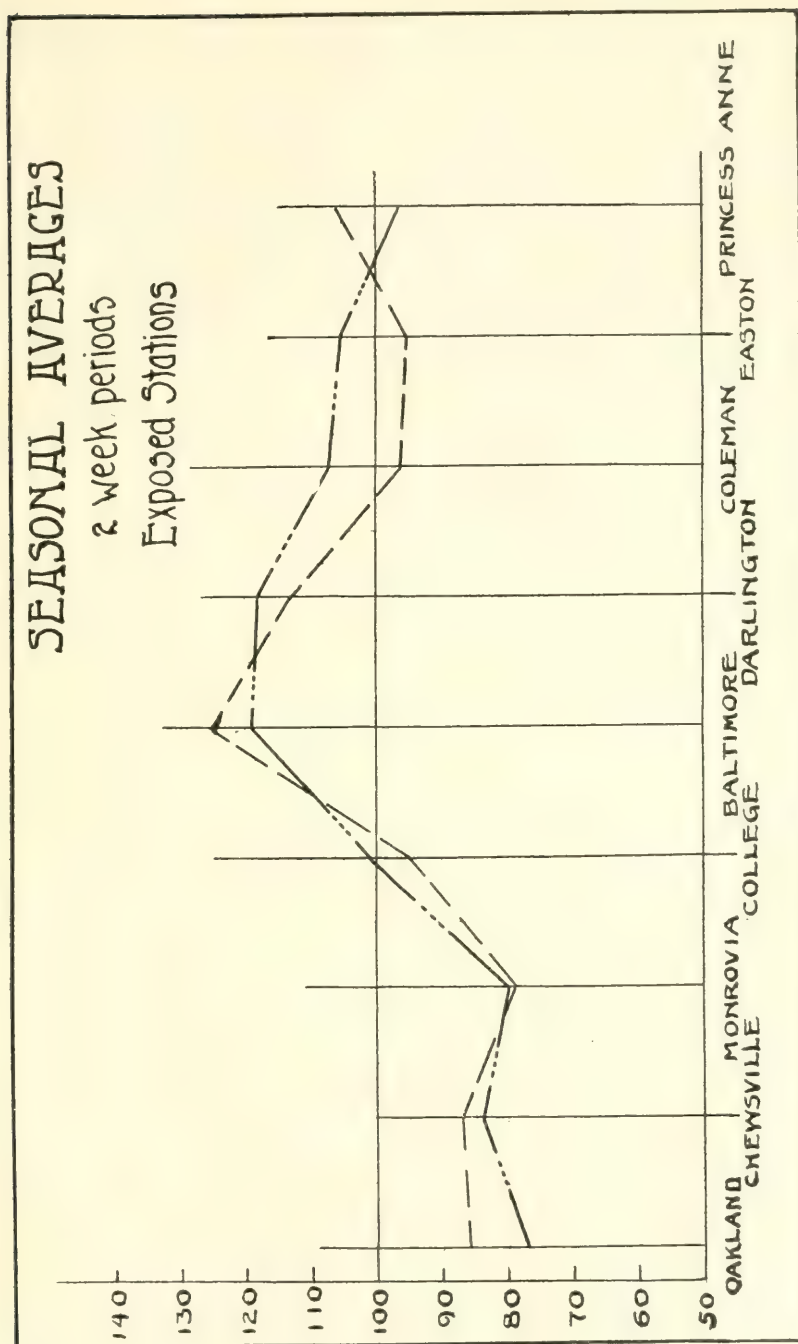


Fig. 7. Seasonal averages of 2-week data, exposed stations.

The 2-week seasonal averages of the two growth measurements taken for the nine exposed stations, represented graphically in figure 7, show that the plant-producing power of the climatic complex is about the same whether it is measured by stem height or leaf-product. The range of variation for stem elongation is from 79 (Monrovia) to 125 (Baltimore). In terms of this growth measurement, the average intensity of the Monrovia climatic complex is 63 per cent of that of the corresponding Baltimore complex. Similarly, the leaf-product mean varies from a minimum of 77 (Oakland) to a maximum of 119 (Baltimore); as measured by leaf-product, the mean intensity of the Oakland climate is 65 per cent as efficient as the corresponding mean for the Baltimore climate. Precipitation is of course left out of account here, as in the other considerations of this paper. The nine stations fall into three groups, according to these mean values: Oakland, Chewsville and Monrovia have low relative values, Baltimore and Darlington have high values, and College, Coleman, Easton and Princess Anne have intermediate and similar values. (See the letters L, H and M in table X.)

Turning to the 4-week seasonal averages, as shown in figure 8, it is seen that the graph for stem height agrees very well with the two 2-week graphs just considered. It is also seen that the 4-week graphs for leaf area and dry weight agree in a satisfactory manner. According to these two graphs, the nine stations fall into the following three groups: Oakland, Chewsville, Monrovia and Easton constitute the groups with low values, Darlington is alone in the group with high values, and College, Baltimore, Coleman and Princess Anne make up the group with intermediate and similar values. (See the letters of table X.)

It is to be remembered that the two series of data (2-week and 4-week) refer to the same total time interval. The plants of one series were registering the same climatic conditions as those of the other series; indeed, they were the same plants, for the 4-week measurements were obtained from the same plants as those from which the corresponding 2-week measurements had been secured. The fact that the seasonal averages of the leaf area values (4-week) do not show the same grouping of the stations as do the leaf-product values (2-week) is to be referred to the fact that the plant alters its internal conditions with growth and age. A soy-bean plant exposed two weeks is an entirely different instrument (as far as measuring environmental efficiency is concerned) from the same plant exposed 4 weeks. For this reason it seems desirable that such studies as the present one should be carried out with as short periods of exposure of the standard plants as is feasible. It is somewhat as though the instrument wore out and altered its characteristics with too long exposure. Since it is obviously impracticable to obtain a large number of plants that are approximately alike, excepting as seeds, it seems desirable to begin each observation with new seed (as was done in this investigation), and to take the final readings before the internal condi-

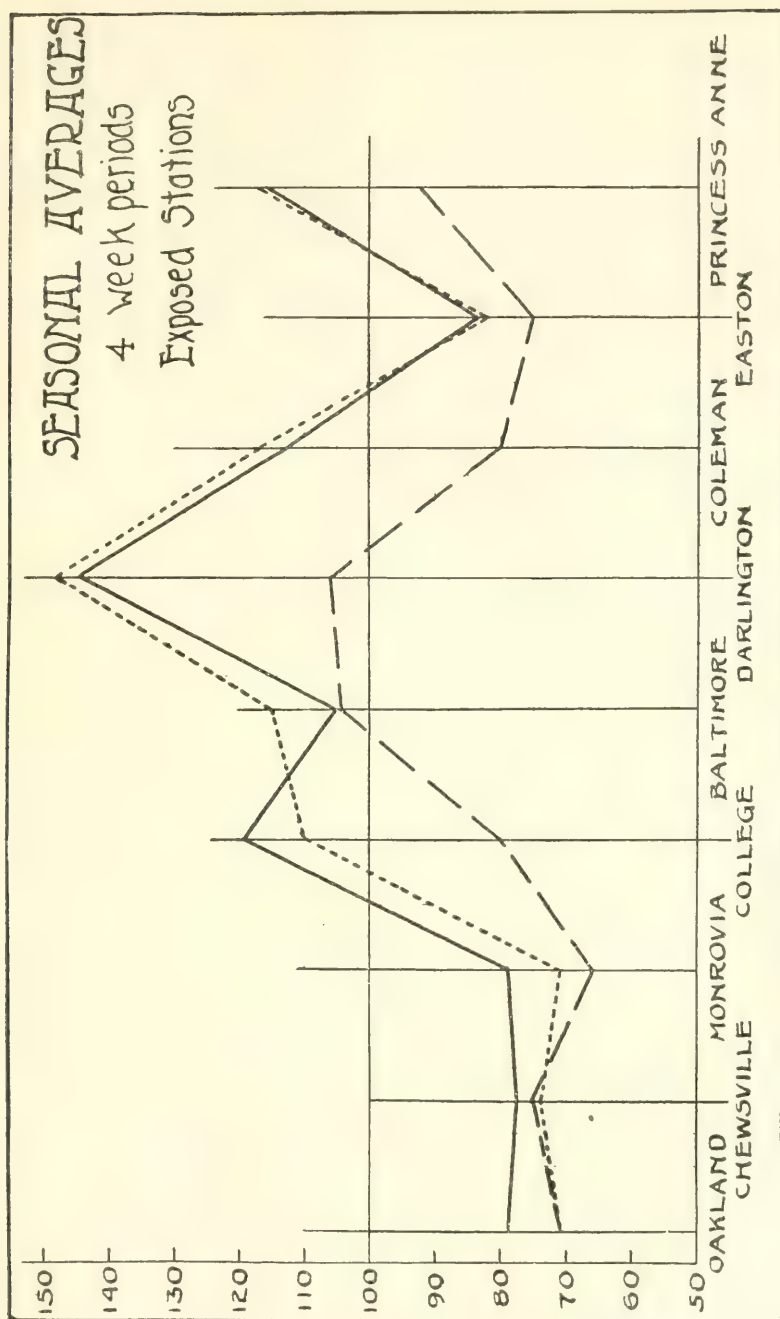


FIG. 8. Seasonal averages of 4-week data, exposed stations.

tions of the plants have been too seriously altered through age and the approach toward maturity. At the same time, the standard plants must of course be allowed to grow long enough so as to be influenced by the fluctuating environmental conditions and long enough to give easily-obtained measurements. McLean (1917) has given some attention to the difference between the behavior of the soy-bean plant during the first and second two weeks of its growth from the seed, under the same set of climatic conditions and fluctuations, pointing out that the plant becomes more sensitive to evaporation conditions as it grows older (since its leaf surface becomes larger). In the use of standard plants as indicators of climatic efficiency the length of time chosen for the exposure period is clearly very important. It may be added that future studies may bring out certain advantages for a 3-week or 4-week exposure of soy-bean plants, as compared with a 2-week exposure, but—as has been pointed out elsewhere in this paper—details will be more apparent when the periods are relatively short, and the principles upon which this sort of work is based are more nearly fulfilled with short periods.

To summarize this discussion, the nine exposed stations arrange themselves in three groups by every one of the five criteria, the grouping is identical by three of the criteria (2-week stem height, 2-week leaf-product and 4-week stem height), it is identical by the two remaining criteria (4-week leaf area and 4-week dry weight), but is it somewhat different by these two separate series of criteria. The differences are: that the second series of criteria place Baltimore in the intermediate instead of in the high group, and Easton in the low instead of in the intermediate group.

It is a striking fact that all five growth criteria agree in placing Oakland, Chewsville and Monrovia in the group for low mean daily values, in giving Darlington high values, and in giving College, Coleman and Princess Anne intermediate values. Only for Baltimore and Easton, among the exposed stations, are there discrepancies.

If the five seasonal values are averaged for each exposed station, the result places Oakland (77), Chewsville (80) and Monrovia (75) in the group for low averages, gives intermediate values for College (101), Coleman (103), Easton (88) and Princess Anne (105), and gives high values for Baltimore (114) and Darlington (126). These average values are shown in the third column of table XI.

The average data for the covered and forest stations, also shown in table X, emphasize the influence of the glass covers and of the forest shade, etc.

It is perhaps important to emphasize that the criterion of stem elongation gives the same grouping of the exposed stations by the 4-week as by the 2-week values. The ratio of the 2-week seasonal mean to the corresponding 4-week mean is shown for each exposed station below.

Oakland.....	1.21	Darlington.....	1.07
Chewsville.....	1.16	Coleman.....	1.20
Monrovia.....	1.18	Easton.....	1.27
College.....	1.19	Princess Anne.....	1.15
Baltimore.....	1.20		

The average of these ratios is 1.18. If, therefore, the stem height of the soy-bean, grown as a standard plant, be used as a measure of the climatic complex, and the measurement be expressed as relative average daily increments, as in this study, the 2-week readings may be approximately reduced to 4-week readings (considering these as the standard) by dividing each 2-week reading by the constant 1.18.

#### THE TOTAL SEASONAL EFFICIENCIES FOR THE SEVERAL STATIONS

The efficiency of an environmental complex, or its power to produce growth in a standard plant, is to be considered as the product of two factors, intensity and duration. If the seasonal averages of the mean daily rates of growth,

TABLE XI

*Relative generalized climatic-efficiency products for the several stations.*

STATION NAME	NORMAL LENGTH OF GROWING SEASON (A)	RELATIVE GENERALIZED SEA- SONAL AVERAGE OF DAILY MEAN INTENSITY (B)	CLIMATIC EFFICIENCY PROD- UCT (AB)
	<i>days</i>		
Oakland.....	117	77	9009
Chewsville.....	156	80	12480
Monrovia.....	—	75	—
College.....	167	101	16867
Baltimore.....	223	114	25422
Darlington.....	188	126	23688
Coleman.....	205	103	21115
Easton.....	201	88	17688
Princess Anne.....	181	105	19005

to which attention has thus far been confined, be taken as the intensity factors for the respective stations, for the season of 1914, and if the length of the entire growing season for each station be taken as the duration factor, or the length of time through which the corresponding intensity is considered as effective, then the product of the length of the season and the corresponding intensity factor should give a value that may approximately represent the relative efficiency of the climatic complex for the station and year in question, by the given plant criterion. Precipitation is of course neglected, as it was not involved in this study.

The seasonal averages of the daily means for the growth rates, as used in this study (table X), may be taken to represent the relative values of the

climatic intensities dealt with, but the lengths of the growing seasons are only approximated by the total lengths of the test periods. Rather than to employ these lengths it will perhaps be better to use the mean (normal) lengths of the growing seasons for the several stations here considered. These may be obtained from Fassig's paper on this subject,<sup>21</sup> and they are shown in table XI, along with the corresponding generalized climatic efficiency products, obtained by multiplying Fassig's mean length of the growing season by the corresponding average climatic intensity (including all five plant criteria) as developed in the preceding section of this paper. It is to be emphasized that the intensity factors are all for the summer of 1914 and that the duration factors are normal, or at least closely approximate normal values.

From table XI it appears that the lowest efficiency product is for Oakland, as would be expected, while the highest is for Baltimore. The Baltimore value is nearly thrice as great as is the value for Oakland. If we regard values above 20,000 as high and those between 10,000 and 20,000 as intermediate, the stations may be grouped as follows:—

Low values: Oakland.

Intermediate values: Chewsville, College, Easton and Princess Anne.

High values: Baltimore, Darlington and Coleman.

These efficiency products may be taken to represent, more or less approximately, the relative values of the climatic conditions at the various stations, to produce plant growth when irrigation is resorted to, so that drought periods are avoided as far as soil moisture is concerned. While there is no reason for thinking that these values (obtained from 2-week and 4-week periods and soy-bean plants, with the particular soil used in this study) may give really quantitative information on these climates as related to plant growth in general, still the product indices here derived are perhaps more reliable than any other series of numerical values that might be readily obtained, and they illustrate a new method by which a beginning may be made aiming toward the quantitative comparison of climatic complexes.

One of the aims of ecological climatology should be to evaluate climates in somewhat the same manner as water-power, mineral deposits, and other geographically restricted sources of power for the accomplishment of human purposes, may be evaluated. The importance of this aim is very great for agriculture and productive forestry, and it is not less important for the fundamental principles of ecology. The above discussion presents one of the first serious attempts to compare the plant-producing powers of several climates by means of numerical indices.

---

<sup>21</sup> Fassig, O. L. The period of safe plant growth in Maryland and Delaware. *Monthly Weather Rev.* **42**: 152-158. 1914.

## GENERAL CONCLUSION

The results and suggestions attained by the study here reported leave the problem of agricultural or ecological climatology still very far from solved, but the purpose of this investigation has been achieved if some of the more fundamental considerations that must be taken into account in this sort of inquiry have been emphasized. The main points brought out are summarized in the Abstract at the beginning of this paper and do not require repetition here. It is clear that this aspect of climatological science required other measures and other methods of treatment than those thus far developed by meteorological climatologists, and that much physiological knowledge must be built into the structure of the new science. It appears that the use of standard plants, in some such way as the soy-bean plants were used in this investigation, and the avoiding of the immense complications due to soil conditions when the same soil is not employed in all cases, will lead to progress in this exceedingly difficult but both fundamentally and practically important field of human advancement. If the relations that hold between climatic conditions and plant growth are to be really understood it will be necessary for the climatological student to interest himself in plant physiology in no merely superficial way, and it will be necessary for much of the science of climatology, as it is now represented in the literature, to be very lightly stressed. The point that seems in need of emphasis is that this new aspect of climatology (or of ecology) will have to deal with climatic conditions *as they affect plants*; it will not need to give main attention to climatic fluctuations and differences *per se*, nor to the meteorological, physical and astronomical reasons for their occurrence.



# THE RELATION OF ACIDITY TO CARBON DIOXIDE ADSORPTION BY CERTAIN GELS AND PLANT TISSUES<sup>1</sup>

CORNELIA LEE CAREY

## ABSTRACT<sup>2</sup>

This paper reports some experiments on adsorption of water, hydrochloric acid and citric acid solutions by agar, gelatine, agar-gelatine and starch gels, coconut endosperm from which oil and soluble matter had been removed, dried cotyledons of *Lupinus albus* (white lupine), *Vicia Faba* (Windsor bean) and *Phaseolus lunatus*, (Lima bean), and also the occlusion of carbon dioxide by agar and gelatine gels, and by agar gels containing either glycocholl, succinic acid, malic acid, or sodium stearate, as well as by dried cotyledons of *Lupinus albus* and *Vicia Faba* and pieces of *Daucus Carota* (carrot) root and *Solanum tuberosum* (potato) tuber. Some of these materials were soaked in water or acid solutions and then exposed to a concentrated atmosphere of carbon dioxide and some of the gels were exposed to carbon dioxide as soon as they had solidified. No citric-acid-soaked material was used in tests on carbon dioxide adsorption. The adsorbing materials were chosen to represent roughly some of the substances characteristic of plant cells, especially of succulents such as cacti. Cactus tissue shows a large daily fluctuation in acidity and exhibits a peculiar respiratory ratio, showing a higher acid content and lower carbon dioxide emission by night than by day. The investigation was undertaken with a view to discover what relation acidity might bear to the possible occlusion of carbon dioxide by the tissues in such plants.

The materials were placed in water or a solution of hydrochloric or citric acid for twenty-four hours and then those employed for gas adsorption were exposed to an atmosphere of carbon dioxide for a like period of time. The carbon dioxide adsorbed was set free by heating and estimated by precipitation in barium hydroxide solution (Pettenkofer method). The amount of water or acid adsorbed was calculated from the increase in weight of the material and from decrease in the weight of liquid in which it had been soaked, and the amount of acid taken up was calculated by determining the acidity of the remaining solution by titration.

The main results of the experiments are as follows:

1. The amount of water adsorbed varies with the substance and with the concentration of the acid employed.

<sup>1</sup> Contribution from the Department of Botany, Columbia University, no. 320.

<sup>2</sup> This abstract was preprinted, without change from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 2, no. 9, May, 1923

2. Agar gel swells more in pure water than does gelatine, but gelatine swells more in acid solutions than does agar. Similar results were obtained by MacDougal.

3. Gelatine shows an apparent reverse in the order of water adsorption in hydrochloric and citric acid solutions. Strengths of acids N/20, N/10, N/5, and N/2 were employed. The greatest water uptake occurred in N/20 hydrochloric acid and the least in N/2, whereas the reverse was true of citric acid. This is probably related to the hydrogen-ion concentration, which is about the same for N/20 hydrochloric and N/2 citric acid. Agar-gelatine acts like gelatine, but not to such a marked degree, due to the presence of the agar. Other materials do not show this sensitivity to hydrogen-ion concentration in the concentrations used.

4. Increase in acid concentration causes increase in weight of acid adsorbed per gram of substance, in both hydrochloric and citric acid solutions.

5. Hydrochloric acid has no marked effect on the adsorption of carbon dioxide by agar. The data suggest that the carbon dioxide occluded decreases as the concentration of acid increases, but the differences are very slight. Citric acid solutions were not tested.

6. Sodium stearate in the agar gel causes greater carbon dioxide adsorption than occurs in pure agar gel. This may have been due to hydrolytic dissociation of the sodium stearate.

7. Gelatine gel adsorbs more carbon dioxide after soaking in water and still more after soaking in hydrochloric acid solution, than the fresh gel does. This is related to the extra water taken up by the gel on soaking, as the gel adsorbs more water in acid solution than in pure water.

8. More carbon dioxide is adsorbed by samples of plant tissue that have been soaked in water than by similar samples that have been in N/10 hydrochloric acid.

9. A few experiments with carbon dioxide adsorption by agar containing organic acids were attempted. These are not complete, so no definite conclusions can be drawn as yet.

10. From these results, as related to gas interchange in plants, especially the cacti, it appears doubtful that increased acidity in the tissues would cause increased ability in the cells to hold carbon dioxide. But the results of this study do not bear directly on this question.

## INTRODUCTION

The tissues of succulent plants such as *Bryophyllum* and the cacti are much more acid in content in the morning than at night. Heyne<sup>3</sup> was the first person to notice this difference in acidity, when he discovered that *Bryophyllum* leaves were acid to the taste in the morning, but that this characteristic

<sup>3</sup> Heyne, B., Zusatz von H. F. Link, ueber die Desoxydation der Blätter von *Cotyledon calycina* B. Heyne, u. einem Beitrag von A. P. Lambert. Trans. Linn. Soc. 41: 213-215. 1815.

disappeared as the day advanced. Link<sup>4</sup> later confirmed these observations by actual tests of the acidity at different times of day, with litmus paper. Several writers have dealt with the question of the influence of acidity upon the power of the tissues to adsorb water. Amongst these is Fischer,<sup>5</sup> who accounts for the swelling produced in œdemas as circulatory disturbances producing abnormal amounts of carbonic and other acids. He supposes that these acids increase the hydration capacity of the colloids and thus bring about œdema.

It has been proposed that acidity might play a considerable part in the power which the tissues possess of adsorbing carbon dioxide. If this is so it might throw some light on certain peculiarities of succulent plants with respect to the interchange of carbon dioxide and oxygen. It was with this idea in mind that the following study was undertaken. To secure, if possible some quantitative data on the influence of acidity on carbon dioxide occlusion, certain plant tissues and some representative colloidal gels were employed. The plan of the work was to investigate the question of water and acid adsorption by the gels and plant tissues and then to determine the effects, if any, which acids might have on the uptake of carbon dioxide by the substances. The work is by no means concluded, and there are many questions which, it is hoped, may be settled in the future.

Strips of prepared colloidal gels of agar, gelatine, agar-gelatine mixtures and starch were studied. Citric and hydrochloric acid solutions were used, of the following normalities: N/20, N/10, N/5, and N/2. Pure water was also used. The gels were selected as in a measure simulating the materials found in living plant cells. Dried cotyledons of *Lupinus albus* (white lupine) *Vicia Faba* (Windsor bean) and *Phaseolus lunatus* (Lima bean), as well as shredded coconut (the endosperm of *Cocos nucifera*) were employed. The last-mentioned material had practically all other substances extracted, so that it was almost pure cellulose. Later a number of materials were studied with regard to their ability to take up carbon dioxide from a very concentrated atmosphere of that gas. These materials were agar and gelatine gels and the cotyledons mentioned above (except *Lupinus*), also sodium stearate and gels of sodium stearate and agar, and pieces of *Solanum tuberosum* (potato) tuber and *Daucus Carota* (carrot) root. Hydrochloric, malic, succinic and amino-acetic (glycocoll) acids were used. The following account of experiments and results is presented under two headings: I, Adsorption of water and acids; II, Adsorption of carbon dioxide.

## I. ADSORPTION OF WATER AND ACIDS

*Agar*, a carbohydrate material obtained from certain sea-weeds, is very largely composed of pentosans, which are also found in higher plants as well.

<sup>4</sup> Link, H. F., On the deoxidation of the leaves of *Cotyledon calycina*, in a letter to A. B. Lambert, Esq. *Jahrb. für Gewächskunde* 12: 73-76. 1820.

<sup>5</sup> Fischer, Martin H., *Oedema and nephritis*. New York, 1915.

especially the cacti. On this account agar was chosen as a typical plant material in the study of the adsorption of acid, gas and water. Gels were made approximately 7.5 per cent; i.e., 7.5 g. of agar and 100 cc. water. The agar was "dissolved" in hot water and the liquid was poured into shallow pans and allowed to harden. This took place rapidly so that it could be cut into strips after standing about 20 minutes at room temperature. The dimensions of the strips were about  $2.5 \times 8 \times 0.1$  cm.

*Gelatine*, an amino-acid compound, may be considered as roughly representing some of the organic nitrogenous compounds of protoplasm. The gelatine gels were 22.5 per cent. The dry gelatine was first soaked in a portion of the required water, after which it was put into the rest of the water which had been heated. Heating was continued only long enough to make the mixture homogeneous, and the resulting hot liquid was poured into pans and when hard cut into strips the same size as those made of agar. The gelatine used was "Cox's," and for carbon dioxide adsorption "Bacto" gelatine.

The agar-gelatine mixture used was a 7.5 per cent gel containing equal parts by weight of agar and gelatine; i.e., 3.25 g. of agar and 3.25 g. of gelatine in 100 cc. water. The agar was prepared as described above, and the gelatine was soaked in a portion of the water and was added after the agar had been "dissolved." The gel was cut into strips as mentioned above for agar and gelatine. Mixtures such as this were first used by MacDougal<sup>6</sup> who employed them as nitrogenous pentosan mixtures simulating, to a degree, swelling reactions such as those of cactus tissue. They were employed in the present experiments for the same reason, although in the proportions used they contained a much higher nitrogenous content than that found in plant tissues.

*Starch gel* was employed to represent a carbohydrate material commonly found in plant cells, though in somewhat a different form. This gel was prepared by autoclaving a mixture of 100 g. of commercial corn starch (Duryeas) and 150 cc. of water. The dry starch was placed in cold water and the mixture was then heated to 105–110°C. for 30 minutes, as previously described by the writer.<sup>7</sup> The size of the dry strips averaged about  $4.9 \times .9 \times 0.1$  cm.

*Coconut endosperm* shredded and extracted (from the seed of *Cocos nucifera*) was used. The brand was "Dromedary", shredded, containing according to the label, sugar, glycerine and salt. It was boiled in a number of waters to free it from these substances, and then placed in running water till it gave almost no test for reducing sugars. By boiling, part but not all of the oil was removed. The dried material was extracted in ether and microscopic sections were studied to determine when all the visible oil was removed. This

<sup>6</sup> MacDougal, D. T., Imbibitional swelling of plants and colloidal mixtures. *Science* 44: 502-505. 1916.

<sup>7</sup> Carey, Cornelia L., A method of preparation and some properties of a starch gel. *Bull. Torrey Bot. Club.* 47: 455-463. 1920.

material consisted almost entirely of cellulose when the extraction was complete. The size of the dry coconut strips varied, particularly in length; those measured averaging  $3.7 \times 0.1 \times 0.1$  cm.

The *cotyledons* used were from seeds of white lupine, Windsor bean and lima bean. The seeds were soaked in water and the epicotyls and hypocotyls, as well as the seed coats, were removed. They were then oven dried and finally brought to constant weight in a calcium-chloride desiccator. Cotyledons only were employed in order to avoid breaking the rest of the embryo in the course of the experiment and thus losing part of the material.

All the materials used for acid and water adsorption were dried to constant weight over calcium chloride, and all but gelatine were oven dried before being placed in the desiccator. The gelatine strips melted at the temperature of the oven. Their first drying was, therefore, at room temperature, 22–25°C.

The strips of starch gel became yellow with age, but the reasons for this change were not studied. The original starch gave no test for reducing sugars, and no acids were evident in the water in which a strip of starch gel had been boiled. A slight trace of reducing sugar was observed in a solution of N/10 hydrochloric acid in which starch strips had remained 24 hours. Water in which starch strips had remained for that period showed no evidence of acid. Hence, no acid diffused out of them with this treatment.

It was found that the gelatine strips liquified when standing in hydrochloric acid solutions at room temperature. The bottles were kept, therefore, in coils of lead pipe through which cool water was flowing. The average temperature of these bottles was about 17°C., at which temperature serious softening did not occur.

The agar-gelatine strips also became soft on standing in acid solutions and could not be treated in the usual manner. The experiments were therefore carried out in flasks, from which the strips were not removed, the alcohol and ether being poured on and off the strips while they were still in the flasks. By this method the strips were not broken as much as would have been the case had they been removed from the container. The ether vapor was removed by suction.

All substances gained in weight while in the desiccator, due to the vapor pressure of the calcium chloride used. Acid diffused out of all substances except agar, starch and coconut endosperm when kept for twenty-four hours in distilled water.

Strips of gels or other substances used were weighed, and all but those of agar-gelatine were put in bottles with ground glass stoppers, containing 100 cc. of acid solution or distilled water. They were then left for 24 hours at a temperature of 22–24°C. At the end of this time the material was taken from the bottles, the excess water was removed by filter paper, followed by quickly dipping into absolute alcohol and ether to remove any water remaining on the outside of the material. It was then again dried with filter paper

and put immediately into a stoppered weighing bottle and weighed. This was done as quickly as possible, to avoid evaporation or extraction of water or adsorption of ether or alcohol by the strips. The acid solutions remaining in the bottles were finally titrated with N/10 sodium hydroxide.

The calculations for acid and water adsorbed from acid solutions (tables III, IV, V, VI) were made as follows: The weight of the material, after soaking in the solution, was subtracted from the original weight, thus giving the actual weight adsorbed by the material. This value was then subtracted from the weight of 100 cc. of the acid solution employed. By multiplying the titration figure obtained after adsorption by the weight of acid in 1 cc. of solution, the weight of acid in 1 cc. of remaining solution is obtained, and multiplying this figure by the weight of solution left after adsorption gives

TABLE I

*Average weight of acid given out and water adsorbed per gram of dry substance, during 24 hours in 100 cc. of water*

	DRY WEIGHT OF MATERIAL USED	ACID GIVEN OUT IN TERMS N 10 NaOH	WATER TAKEN IN
	g.	g.	g.
Cotyledons, Lima bean.....	2.095	0.0072	1.128
Cotyledons, lupine.....	2.064	0.0046	1.485
Cotyledons, Windsor bean.....	2.214	0.0044	1.251
Agar-gelatine.....	2.557	0.0012	7.125
Gelatine.....	2.694	0.0012	3.818
Starch.....	1.667	None	0.916
Coconut endosperm.....	0.410	None	4.54
Agar.....	1.384	None	7.77

the actual amount of acid in the solution. This figure subtracted from the weight of acid in the original solution gives the weight of acid adsorbed. By subtracting this figure from that of the gain in weight in the material due to adsorption the amount of water taken up is obtained, and from these two figures (i.e., the acid and water adsorbed) the weight imbibed per gram may be easily calculated.

All the substances used were tested for acid diffusing out when in distilled water. The following table (table I, also table VII) gives the weight lost by diffusion per gram of substance, and also the weight of water adsorbed per gram. It appears that the carbohydrates do not lose any acid when in water, but gelatine, agar-gelatine (due to the gelatine it contains) and the cotyledons do. The acid lost may very well be a mixture of acids including amino acids, though no test was made to determine the composition of the mixture.

The greater adsorption by agar-gelatine than by gelatine is indubitably due to the presence of agar, which swells more in pure water than gelatine

does. MacDougal<sup>8</sup> observed this in comparing the swelling of pure agar and gelatine with mixtures of the two. As the proportion of the agar was increased the swelling in water likewise increased, the greatest enlargement being with pure agar.

The acid diffusion from materials should take place until equilibrium is reached between the acid concentration in the surrounding solution and that within the material. No attempt has been made to determine just what acids diffuse out or to ascertain the H-ion concentration of the solution. In tables V and VI, a correction has been made for this diffusion based on the constant obtained by standing the material in water (table VII). This is probably not absolutely correct as diffusion would be altered when the substance is placed in acid solutions. If it is a function of the H-ion concentration then the stronger acid would probably displace the weaker and, other things being equal, diffusion in an acid medium would then be greater than that in pure

TABLE II

*Adsorption of fresh 10-per cent gelatine gel in 100 cc. of water or of N/10 hydrochloric acid, in grams in 24 hours*

IN WATER		IN N/10 HYDROCHLORIC ACID	
Weight of gelatine used	Gain in weight	Weight of gelatine used	Gain in weight
<i>g.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>
15.23	2.91	14.95	11.58
14.60	2.10	15.00	10.70
13.94	2.11	13.42	11.00
Total, 14.65	2.07	14.45	11.09

water. This would make the apparent adsorption of acid really more than it is, even with the correction. It is not impossible that the views of Donnan<sup>9</sup> and Harris,<sup>10</sup> as to the permeability of membranes might be applicable to these substances. These writers put forward the view that membranes that are ordinarily perfectly permeable to salts may be markedly influenced by the presence of an electrolytically dissociated, non-dialysing colloidal substance (such as congo red or protein in the case of a living cell) on one side of the membrane, so that the permeability in one direction may almost disappear.

From the experimental data (table V) it appears that the more concentrated the acid the more is adsorbed per gram of substance, and, comparing any two similar concentrations, the actual weight of citric acid adsorbed by the same material is greater than that of hydrochloric. The amount taken up in the same concentration varies with the substance; for example, coconut

<sup>8</sup> *Loc. cit.*, see note 6.

<sup>9</sup> Donnan, F. G., Theorie der Membrangeleichgewichte und Membranpotentiale bei Vorhandensein von nicht dialysierenden Elektrolyte. *Zeitschr. Elektro-chem.* 17: 572-581. 1911.

<sup>10</sup> Donnan, F. G., and L. A. B. Harris, The osmotic pressure and conductivity of cationic solutions of congo reds, and reversible membrane equilibria. *Trans. Chem. Soc.* 99: 1554-1577. 1911.

endosperm adsorbs more acid than does either agar or starch in the same strength of acid—the order of imbibition being greatest with coconut, less with agar and least with starch. Neither does the additional weight of acid imbibed with higher concentrations form a definite ratio; for instance, agar in N/20 hydrochloric acid takes up 0.0133 g. per gram of substance and in citric acid N/20 it takes up 0.0277 g., whereas in N/2 hydrochloric acid it takes up 0.0689 g. as against 0.2040 g. in citric acid,—the imbibition being five times as great in N/2 hydrochloric as in N/20, and seven times in citric acid. Coconut endosperm in N/20 hydrochloric acid adsorbs 0.0234 g. per gram of material and in N/20 citric acid 0.0525 g., whereas in N/2 hydrochloric and citric acids the corresponding figures are 0.0902 and 0.3025 g., respectively,—about three and a half in hydrochloric acid to six times as much in citric acid. The acid absorption by starch is less than that by any other substance tested, being 0.0026 g. in N/20 hydrochloric acid and 0.0119 g. for the same concentration of citric acid, and for N/2 0.0144 and 0.0813 g., respectively. The figures for the cotyledons are about the same as those for starch, varying somewhat with the kind of cotyledons. The lupine values run from 0.0036 to 0.0371 g. per gram in hydrochloric, and from 0.0067 to 0.0932 g. in citric acid. The Lima bean values run from 0.055 to 0.0273 g. for hydrochloric acid and from 0.0057 to 0.0720 g. for citric acid, while the Windsor bean values are slightly higher, the figures being 0.0083 g. in N/20 and 0.0276 g. in N/2 hydrochloric acid; in citric acid they run from 0.0086 g. in N/20 to 0.0864 in N/2. Gelatine takes up more acid by weight per gram of substance than any other material used, adsorbing 0.0402 g. in N/20 hydrochloric acid and 0.0641 g. for the same strength of citric acid. In the half-normal acids the weight increment is 0.1095 g. in hydrochloric acid and 0.5197 g. in citric acid,—an increase of about two and a half times as much in hydrochloric acid to eight times in citric acid. Agar-gelatine gel imbibes from 0.0184 to 0.0759 g. in N/20 and N/2 hydrochloric acid, respectively, and from 0.0409 to 0.2411 g. in the same strengths of citric acid. It is to be noted that these adsorption values, both for water and for acids, are only relative, the actual weights depending on the previous history of the material employed and upon other variables.

The percentages of both acid and water adsorbed from the original acid solutions were calculated for all materials in both hydrochloric and citric acids. By making a ratio of the percentage of acid to water imbibed from a solution by any one substance, it was found that the ratio approached unity (with some variations in certain substances) as the concentration of the solution increased. In general, in weaker solutions proportionally more acid was removed than water from the solution, and the solutions were in all cases less acid after than before the material had been placed in them. The data at hand at present are not sufficient to come to any definite conclusions on differential adsorption, and the writer is not yet prepared to say how

much weight should be given to the apparent fluctuations that occur in the acid-water ratios of some of the materials such as the cotyledons and starch. Further work is planned. As to the average percentage of acid imbibed from solutions per gram of substance, gelatine adsorbs the most, taking up 22 per cent of acid in N/20 hydrochloric acid solution, and 14.4 per cent of water from the same solution, while it takes up 6 per cent of acid per gram, and 5 per cent of water, in N 2 hydrochloric acid solution. Coconut endosperm takes up 12.8 per cent of acid in N 20 and 4.9 per cent in N 2 hydrochloric acid, while it takes up 5.9 and 5.7 per cent of water, respectively, from the same concentrations. Starch adsorbs least from hydrochloric acid solutions, only 1.4 per cent of acid in N/20 and 0.8 per cent in N 2 is imbibed per gram of material, while the water taken up is 0.8 per cent for both N 20 and N 2 hydrochloric acid solutions. The agar figures fall from 7.3 per cent acid per gram of substance in N/20, to 3.8 per cent in N/2 hydrochloric acid, and from 4.3 per cent to 3.5 per cent of water, respectively, in the same concentrations. The figures for citric acid solutions are much the same as those for hydrochloric acid solutions. Gelatine imbibed 18.3 per cent of citric acid per gram in N 20 citric acid solution, and 14.8 per cent in N 2 citric acid solution. The water taken up in the two solutions was 7.4 and 12.1 per cent, respectively, per gram of material, more water being taken up in the N 2 than the N 20 citric acid solution. Coconut endosperm adsorbs 15 per cent, and 8.6 per cent of citric acid per gram in N 20 and N 2 solutions, while it took up 5.4 per cent and 5.3 per cent of water, respectively, from the same solutions. Agar-gelatine took up 14.7 per cent of acid per gram in N/20 citric acid and 6.9 per cent in N/2 acid. It took up 3.7 per cent of water in N/20 and 4.8 per cent in N/2 citric acid. The water adsorbed in these acid solutions increases with the acid concentration of the solution, as is the case with gelatine in citric acid. This is undoubtedly due to the gelatine that the mixture contains. Agar adsorption runs from 7.9 to 5.8 per cent of acid per gram in N 20 and N 2 citric acid, respectively, and from 5.6 to 5 per cent, of water for the same concentrations. Starch takes up 3.4 per cent of acid in N 20 and 2.3 per cent, in N 2 citric acid per gram of substance, and 0.9 and 0.8 per cent, of water, respectively, from the same concentrations. The cotyledons do not show as low percentages of adsorption in hydrochloric acid as does starch, but take up less acid than starch does in citric acid solutions. Both the cotyledons and starch show certain irregularities having maximum percentage adsorptions in some of the more concentrated acids, which need further investigation.

The question may arise as to the advisability of comparing the adsorption of substances such as agar strips with shredded coconut endosperm, since the surface exposed varies considerably. Complete adsorption may have taken place in one substance and not in another, in the same length of time. The tests were not taken up with an idea of obtaining complete adsorption.

TABLE III

*Average adsorption of hydrochloric acid and water from hydrochloric acid solutions, in grams per gram of substance, in 24 hours; also, for agar and  $\frac{N}{10}HCl$ , for from 2 to 14 days*

ACID CONCENTRATION	NUM- BER OF DETER- MINA- TIONS	ORIGINAL WEIGHT (USED)	GAIN	N/10 NaOH TO NEU- TRALIZE 10 CC. OF SOLUTION	ACID AB- SORBED PER GRAM	WATER ABSORBED PER GRAM
		g.	g.	cc.	g.	g.
Agar:						
N 20.....	14	6.603	24.362	3.84	0.0116	3.692
N 10.....	8	7.142	15.553	8.13	0.0159	2.164
N 5.....	6	6.656	23.493	18.47	0.0319	3.497
N/2.....	8	4.543	16.550	48.44	0.0699	3.571
Starch:						
N 20.....	6	5.999	10.891	4.82	0.0026	0.822
N 10.....	6	6.920	5.581	9.69	0.0044	0.804
N/5.....	4	6.109	4.789	19.57	0.0078	0.786
N/2.....	6	5.775	4.726	49.79	0.0144	0.812
Coconut endosperm:						
N 20.....	6	1.026	6.036	4.61	0.0234	5.884
N 10.....	6	1.513	8.978	9.35	0.0359	5.890
N 5.....	6	1.016	5.785	19.73	0.0479	5.645
N/2.....	6	1.018	5.857	50.04	0.0902	5.678
Agar-gelatine:						
N/20.....	10	6.683	30.132	2.33	0.0184	4.522
N/10.....	10	7.437	30.428	7.34	0.0238	4.067
N/5.....	8	6.524	25.171	18.24	0.0332	3.823
N 2.....	8	3.581	13.712	49.01	0.0759	3.914
Gelatine:						
N 20.....	6	2.689	38.704	3.31	0.0402	14.357
N 10.....	6	2.536	24.386	8.23	0.0548	9.829
N/5.....	6	2.712	15.965	17.69	0.0686	5.893
N/2.....	6	2.437	12.420	48.23	0.1095	5.022
Vicia cotyledons:						
N 20.....	6	6.131	6.796	7.73	0.0083	1.101
N 10.....	6	6.250	7.388	8.00	0.0150	1.167
N 5.....	6	6.140	6.990	17.50	0.0217	1.117
N 2.....	6	6.114	6.776	48.18	0.0276	1.081
Phaseolus cotyledons:						
N/20.....	6	4.199	4.960	4.60	0.0055	1.176
N 10.....	6	4.168	5.007	8.96	0.0129	1.189
N 5.....	6	4.222	5.405	18.67	0.0197	1.260
N 2.....	6	4.160	5.174	48.93	0.0273	1.217
Lupinus cotyledons:						
N 20.....	6	6.265	8.980	4.81	0.0036	1.426
N/10.....	5	6.173	9.409	8.65	0.0127	1.510
N/5.....	6	6.036	9.204	18.29	0.0202	1.520
N 2.....	6	6.040	9.910	48.16	0.0371	1.604
Agar and N/10 HCl, 2-14 days:						
2 da.....	4	6.405	19.699	8.45	0.0174	2.919
4 da.....	2	6.451	23.957	8.69	0.0191	3.694
7 da.....	3	6.803	18.380	8.86	0.0148	2.689
10 da.....	3	7.031	19.094	9.04	0.0139	2.699
14 da.....	1	7.826	21.482	8.96	0.0137	2.731

TABLE IV

*Average adsorption of citric acid and water from citric acid solutions, in grams per gram of substance, in 24 hours*

ACID CONCENTRATION	NUM- BER OF DETER- MINA- TIONS	ORIGINAL WEIGHT USED	GAIN	N 10 NaOH TO NEU- TRALIZE 10 CC. OF SOLUTION	ACID AB- SORBED PER GRAM	WATER ABSORBED PER GRAM
		g.	g.	cc.	g.	g.
Agar:						
N/20.....	6	4.272	24.155	4.36	0.0277	5.625
N/10.....	6	4.230	25.321	9.16	0.0520	5.932
N/5.....	6	3.854	19.700	18.62	0.0904	5.187
N/2.....	6	4.023	20.554	47.46	0.2040	4.925
Starch:						
N/20.....	6	2.053	1.838	4.74	0.0119	0.879
N/10.....	5	2.195	2.048	9.54	0.0204	0.915
N/5.....	6	1.933	1.797	18.95	0.0478	0.899
N/2.....	6	1.916	1.705	48.01	0.0813	0.814
Coconut endosperm:						
N/20.....	6	1.010	5.492	4.48	0.0525	5.388
N/10.....	6	1.018	5.415	9.18	0.0895	5.233
N/5.....	6	1.013	5.390	18.59	0.1601	5.161
N/2.....	6	1.022	5.648	47.63	0.3025	5.219
Agar-gelatine:						
N/20.....	6	2.613	9.650	3.86	0.0409	3.649
N/10.....	6	2.772	11.175	8.38	0.0643	3.995
N/5.....	6	2.592	11.426	17.64	0.1157	4.294
N/2.....	6	2.614	12.971	46.38	0.2411	4.717
Gelatine:						
N/20.....	6	2.579	18.852	3.30	0.0641	7.418
N/10.....	6	2.733	27.920	7.50	0.1197	10.227
N/5.....	7	2.911	29.103	16.15	0.2075	10.008
N/2.....	6	2.651	32.506	44.20	0.5197	11.820
Vicia cotyledons:						
N/20.....	6	4.105	4.740	4.71	0.0086	1.146
N/10.....	5	4.134	4.730	9.13	0.0218	1.122
N/5.....	5	4.344	4.796	18.28	0.0404	1.066
N/2.....	6	4.147	4.633	46.41	0.0864	1.032
Phaseolus cotyledons:						
N/20.....	6	4.157	4.839	4.93	0.0057	1.159
N/10.....	6	4.080	4.681	9.55	0.0150	1.132
N/5.....	6	4.155	4.676	18.99	0.0308	1.095
N/2.....	6	4.251	4.829	47.28	0.0720	1.064
Lupinus cotyledons:						
N/20.....	6	4.040	5.877	4.89	0.0068	1.448
N/10.....	6	4.037	5.785	9.36	0.0197	1.413
N/5.....	4	4.041	6.017	18.65	0.0412	1.448
N/2.....	6	4.053	6.007	46.78	0.0932	1.389

but rather that in a stated time, and the amounts of material adsorbed in different substances are not compared but the amounts taken up by one substance in different acids and in various concentrations are. Twenty-four hours was taken as an arbitrary time standard, since in this time it was assumed that no bacterial action would take place in any liquids in which this might occur, and that the substances would not disintegrate on remaining this length of time in the liquids. It is doubtless true that the surface exposed and the time play an important part in bringing about adsorption equilibria, and it is also surely true that, other things being equal, the greater the surface the more rapidly this equilibrium will be approached. No tests were made to determine any equilibria except in the case of agar strips. A few strips of this substance were placed in N/10 hydrochloric acid for varying lengths of time (table III). Amongst these the greatest acid adsorption was after four days, as was also the greatest water adsorption from the same solution. From this point on to two weeks both the acid and water adsorption decreased, with the exception of the water taken up in fourteen days, which was slightly greater than that after ten days. The cause for this decrease in imbibition is not known. It may have been due to some disintegration or diffusion from the strips, which was not apparent. These results, however, were taken from only a few experiments, so they cannot be considered conclusive.

It will be noticed (tables I and VI) that some substances take in more water from pure water than from citric and hydrochloric acid solutions; these are agar, agar-gelatine and starch gels and the *Vicia* cotyledons. Those adsorbing more water from hydrochloric acid solutions than from pure water are coconut endosperm, gelatine and the cotyledons of lupine and Lima bean. In citric acid solutions the gelatine imbibes more water than when in pure water. The reverse is the case with *Lupinus* cotyledons in citric acid, whereas *Phaseolus* cotyledons vary, some showing higher water intake in acids (namely in N/20 and N/10 citric acid), whereas those in N/5 and N/2 imbibe less water than do those in pure water. The cause of this is not clear. If it is a simple question of carbohydrate material as opposed to material containing organic nitrogen, one would expect that coconut endosperm would be in the group with agar and starch, but this is not the case. So, also, one would expect to find all the cotyledons in the same group with the carbohydrates, since they contain such a large proportion of these materials and a mixture of agar and gelatine containing equal parts by weight of the two substances acts as agar and starch do.

In considering the water adsorption (table VI, also III and IV), except for gelatine, which will be discussed later, the difference in weight imbibed by any one substance in different strengths of the same acid is not very marked, the curves being practically flat. Comparing the carbohydrates, more water is adsorbed in citric acid than in hydrochloric acid of the same normality. This is also true of starch, which adsorbs very little water.

TABLE V  
Comparison of amounts (grams) of hydrochloric acid and citric acid adsorbed from solution, per gram of substance, in 24 hours

	WITHOUT CORRECTION							WITH CORRECTION							
	Agar A	Agar B	Agar C	Starch	Coconut endosperm	Agar-gelatin mixture	Gelatin	Lupinus cotyledons	Viola cotyledons	Phaseolus cotyledons	Agar-gelatin mixture	Gelatin	Lupinus cotyledons	Viola cotyledons	Phaseolus cotyledons
HCl N 20.....	0.0116	0.0118	0.0133	0.0026	0.0234	0.0184	0.0402	0.0036	0.0083	0.0055	0.0196	0.0414	0.0082	0.0127	0.0127
Citric acid N/20.....	—	—	0.0277	0.0119	0.0525	0.0409	0.0641	0.0068	0.0086	0.0037	0.0421	0.0653	0.0114	0.0130	0.0129
HCl N/10.....	0.0159	—	0.0215	0.0044	0.0359	0.0238	0.0548	0.0127	0.0150	0.0129	0.0250	0.0569	0.0173	0.0194	0.0201
Citric acid N/10.....	—	—	0.0520	0.0204	0.0895	0.0643	0.1197	0.0197	0.0218	0.0150	0.0655	0.1209	0.0243	0.0262	0.0222
HCl N/5.....	—	0.0319	0.0346	0.0078	0.0479	0.0332	0.0686	0.0202	0.0217	0.0197	0.0344	0.0698	0.0248	0.0261	0.0269
Citric acid N 5.....	—	—	0.0904	0.0478	0.1601	0.1157	0.2075	0.0412	0.0404	0.0208	0.1169	0.2087	0.0458	0.0448	0.0380
HCl N 2.....	—	0.0699	0.0890	0.0144	0.0902	0.0759	0.1095	0.0371	0.0276	0.0273	0.0771	0.1107	0.0417	0.0320	0.0345
Citric acid N 2.....	—	—	0.2010	0.0813	0.3025	0.2411	0.5197	0.0932	0.0864	0.0720	0.2423	0.5209	0.0978	0.0908	0.0792

TABLE VI  
Comparison of amounts (grams) of water adsorbed from hydrochloric and citric acid solutions, per gram of substance, in 24 hours

	WITHOUT CORRECTION							WITH CORRECTION							
	Year A	Year B	Year C	Starch	Coconut endosperm	Agar-gelatin mixture	Gelatin	Lupinus cotyledons	Viola cotyledons	Phaseolus cotyledons	Agar-gelatin mixture	Gelatin	Lupinus cotyledons	Viola cotyledons	Phaseolus cotyledons
HCl N 20.....	3.672	4.242	4.272	0.822	5.884	4.522	14.357	1.426	1.101	1.176	4.521	14.356	1.421	1.097	1.169
Citric acid N 20.....	—	—	5.625	0.879	5.388	3.649	7.418	1.448	1.146	1.159	3.648	7.417	1.443	1.142	1.152
HCl N 10.....	2.164	—	3.917	0.804	5.890	4.067	9.829	1.510	1.167	1.189	4.066	9.828	1.505	1.163	1.182
Citric acid N 10.....	—	—	5.932	0.915	5.233	3.995	10.227	1.413	1.122	1.132	3.994	10.226	1.408	1.118	1.145
HCl N 5.....	—	3.497	3.570	0.786	5.645	3.823	5.893	1.520	1.117	1.260	3.822	5.892	1.515	1.113	1.253
Citric acid N 5.....	—	—	5.187	0.899	5.161	4.294	10.008	1.448	1.066	1.095	4.293	11.007	1.443	1.062	1.088
HCl N 2.....	—	3.571	3.462	0.812	5.678	3.914	5.022	1.604	1.081	1.217	3.913	5.021	1.599	1.077	1.210
Citric acid N 2.....	—	—	4.925	0.811	5.219	4.717	11.820	1.389	1.032	1.061	4.716	11.818	1.384	1.028	1.057

With coconut endosperm less water is taken up in citric acid. The cotyledons show but very slight differences even throughout the whole series. This is probably due to the mixture of carbohydrates and proteins they contain, the former being present in larger amount. In the case of *Vicia* and *Phaseolus* cotyledons, the carbohydrate is largely starch.

The fact that *Lupinus* cotyledons do not contain starch<sup>11</sup> but other carbohydrates, and also, that their nitrogenous substance is in greater proportion than in cotyledons of either *Phaseolus* or *Vicia*, does not seem to be particularly apparent in the difference in adsorption in the two acids used. This may be due to the fact that in these cases the carbohydrate is far greater in amount than the nitrogenous material.

Dachnowski<sup>12</sup> found that *Phaseolus* seeds swell more and contain more water in solutions of acid than when in water. A maximum was attained above which further increase in concentration led to a diminishing water retention. He concluded that the amount of water retained was determined not by the concentration so much as by the effect of the anions of the particular acid concerned, and also that hydrolytic cleavage takes place in the seeds, whereby the water in them varies and is greater the greater the hydrolysis. His results with *Phaseolus* compare closely with those of the writer, as to the tests with hydrochloric acid, but not with the tests here reported with citric acid, the water content increasing slightly in the former as the concentration increases and decreasing in the latter. Dachnowski used whole seeds, so the two experiments should not be compared too closely. Shull,<sup>13</sup> working on water intake in seeds, came to the conclusion that during the first part of adsorption the water intake is very rapid. The velocity being approximately an inverse exponential function of the total preceeding adsorption. He obtained these results with seeds of *Xanthium Pennsylvanicum* and three varieties of peas.

Very little has been done on adsorption by either pure starch or cellulose. According to Lloyd,<sup>14</sup> the adsorption of hydrochloric acid, sodium hydroxide and sodium chloride varies with different starches, but not as much as would be expected considering the size of the particles. The adsorption is greatest in the hydroxide solution.

In 1916 Leighton<sup>15</sup> published some results on adsorption by cotton fibers, showing that neither hydrochloric, sulphuric nor phosphoric acid formed compounds with cellulose. Hydrochloric acid is adsorbed least of the

<sup>11</sup> Schuize, E., E. Steiger, and W. Maxwell, Untersuchung über die chemische Zusammensetzung einiger Leguminensamen. Landw. Versuchsstat. 39: 269-326. 1891.—König, K., Chemie der menschlichen Nahrungs und Genussmittel. Vol. I. p. 403. Berlin, 1893.—Blyth, Alex., and Meridith W. Blyth, Foods, their composition and analysis. P. 185. New York. 1903.

<sup>12</sup> Dachnowski, Alfred, The effects of acid and alkaline solutions on the water relation and metabolism of plants. Amer Jour. Bot. 1: 412-439. 1914.

<sup>13</sup> Shull, Charles A., Temperature and the rate of moisture intake in seeds. Bot. Gaz. 69: 361-390. 1920.

<sup>14</sup> Lloyd, Hoyes, Adsorption of some substances by starches. Jour. Amer. Chem. Soc. 33: 1213-1226. 1911

<sup>15</sup> Leighton, Alan, Adsorption of acids by cellulose. Jour. Phys. Chem. 20: 188-194. 1916.

three and sulphuric acid most, while the presence of any one of the three acids cuts down the amount of water adsorbed by the cotton fibers. Selective adsorption was greatest with the hydrochloric acid and did not occur with phosphoric acid. From the work of the writer, starch adsorbs more water from citric than from hydrochloric acid solution of the same strength. The starch imbibes less water than any other substance and its acid imbibition in citric is considerably higher than that in hydrochloric acid, while the curve of the water intake in both acids is quite flat. The reverse is true of coconut endosperm.

If the adsorption of water by gelatine in hydrochloric and citric acid be observed (table VI) it will be noticed that gelatine swells most in N 20 hydrochloric acid for the hydrochloric acid series, and most in N 2 citric acid for that series. That is, with increased concentration we get a fall in adsorption in hydrochloric acid while the reverse is true in citric acid. The real cause for this peculiar relation probably lies in the hydrogen-ion concentration. If we compare the tables of ionization of hydrochloric and citric acid, as compiled by Thomas,<sup>16</sup> it will be seen that N 20 hydrochloric acid contains roughly about the same number of grams of hydrogen-ions per liter as does N/2 citric acid. Agar-gelatine gel shows the same sort of swelling, but to a less marked degree, probably due to the presence of the agar.

Fischer<sup>17</sup> states that the swelling of gelatine in acids is partly dependent on the concentration of the acids, but that gelatine swells more in acids than in pure water.

Ostwald<sup>18</sup> considers swelling dependent in a complicated manner on the concentration. He obtained swelling in very weak acids, such as N 210 hydrochloric acid. This declined toward pure water. With stronger concentrations it increased, reaching a maximum with N 40 hydrochloric acid. From this he considered swelling the exclusive influence of the hydrogen-ions. This appears to agree in general with the results obtained by the writer for the amounts of water adsorbed by gelatin in hydrochloric acid, which fall steadily from N/20 as the concentration is increased.

According to Loeb,<sup>19</sup> acids, alkalies and neutral salts combine with protein in accordance with the chemical forces of primary valency that govern the combining of crystalloids and, since the early workers did not consider the variable hydrogen-ion concentration of the proteins, the Hofmeister series does not really exist. The same quantity of isoelectric gelatine will combine with nitric, oxalic and phosphoric acids in the proportions of 1:2:3. Loeb, however, felt that these experiments had not proceeded far enough to make

<sup>16</sup> Thomas, Arthur W., Tabulation of hydrogen and hydroxyl ion concentrations of some acids and bases. Jour. Amer. Leather Chemists Assoc. 15: 133-146. 1920.

<sup>17</sup> *Loc. cit.*, p. 60, see note 5.

<sup>18</sup> Ostwald, Wolfgang, Über den Einfluss von Säuren und Alkalien auf die Quellung von Gelatine. Arch. Physiol. 103: 563-589. 1905.

<sup>19</sup> Loeb, Jacques, Proteins and colloid chemistry. Science 52: 449-456. 1920.

it possible to say whether the reaction is that of adsorption of the ions or chemical combination.

Procter,<sup>20</sup> working on gelatine came to similar conclusions on this matter. He says swelling of gelatine in water is influenced by the character of the gelatine used. He comes to the same conclusion as Ostwald,<sup>21</sup> and MacDougall<sup>22</sup> also shows this in his work on the swelling of gelatine and of agar-gelatine mixtures in N/100 hydrochloric acid; namely, that gelatine swells more in acids than in pure water. This swelling, Procter states, increases with increased concentration, until finally complete solution takes place in weak acids. With stronger acids there occurs a concentration at which swelling is at a maximum, and higher concentrations give swelling that diminishes in a hyperbolic curve as the acid concentration is increased.

According to Miss Lloyd,<sup>23</sup> the rate of adsorption of dry gelatine in water depends on temperature, hydrogen-ion concentration, and a number of other factors, and Mrs. Shreve,<sup>24</sup> as well as MacDougall,<sup>25</sup> have found that the previous history of the gelatine plates used influences the manner of swelling.

MacDougall<sup>26</sup> placed consisting gels of ninety parts of agar and ten parts of bean protein in N/100 hydrochloric acid, citric acid and sodium hydroxide, and determined the swelling that occurred. His results show that more water was taken up from citric than from hydrochloric acid. This is indubitably due to the fact that carbohydrate predominated in his mixtures, since pure agar in the concentrations employed by the writer took up more water from citric than from hydrochloric acid. As far as the amount of acid is concerned, gelatine actually adsorbs more per gram of substance in citric than in hydrochloric acid, according to the results here reported. The same is true of agar and of a mixture of the two, also of starch, coconut endosperm and the cotyledons tested. From MacDougall's<sup>27</sup> experiments it is evident that proteins have the highest water capacity in acids, whereas pentosans show the greatest degree of hydration in neutral or slightly alkaline solutions.

Borowikow<sup>28</sup> thought acidity a favoring factor for growth, but Long<sup>29</sup> obtained greater swelling in samples of *Opuntia Blakeana* which were less acid. He thought this was due to the direct effect of acids on the colloids of the cell and concludes from this that protoplasm differs from such sub-

<sup>20</sup> Procter, Henry R., Über die Einwirkung verdünnte Säuren und Salzlösungen auf Gelatine. Kolloidechem. Beih. 2: 243-284. 1911.

<sup>21</sup> Loc. cit., see note 18.

<sup>22</sup> Loc. cit., see note 6.

<sup>23</sup> Lloyd, Dorothy Jordan, On the swelling of gelatine in hydrochloric acid and caustic soda. Biochem. Jour. 14: 147-170. 1920.

<sup>24</sup> Shreve, Edith B., Investigations on the imbibition of water by gelatine. Science 48: 324-327. 1918.

<sup>25</sup> MacDougall, D. T., The effects of age and the inclusion of salts on the heterotrophic action of colloidal bodies of cytological interest. Proc. Soc. Biol. and Med. 18: 244-246. 1921.

<sup>26</sup> MacDougall, D. T., The effects of acids and salts on "biocolloids." Science 46: 269-272. 1917.

<sup>27</sup> MacDougall, D. T., Water deficit and action of vitamins, amino-compounds and salts on hydration. Amer. Jour. Bot. 8: 296-302. 1921.

<sup>28</sup> Borowikow, G. A., Ueber die Ursachen des Wachstums der Pflanzen Zeitschr. Kolloide 15: 27-30. 1914.

<sup>29</sup> Long, Esmond R., Growth and colloid hydration in cacti. Bot. Gaz. 59: 491-497. 1915.

stances as fibrin and gelatine, which exhibit marked increase in swelling in acid solutions. From MacDougal's work this relation is probably due to the high pentosan content of the cacti.

Taylor<sup>30</sup> considers adsorption proportional to the actual extension of surface; that is, the adsorptive power of any substance is a function of its specific surface. He also, as do others, considers it as a process tending toward an equilibrium.

The following is a quotation from Taylor on imbibition of gels from solutions or salts and acids. "When a gel undergoes imbibition in solution it takes up both solute and solvent, but in different amounts which depend to different extents on the concentration of the solution. The water taken up increases to a maximum (at about 14 per cent sodium chloride) and then decreases slowly as the concentration increases. The amount of salt taken up increases with the concentration, and always approximately proportionally to the concentration."

"The action of acids and bases on imbibition by gelatine is similar to the action of salts. At small concentrations (0.005–0.01 M) imbibition is less than in pure water, reaches a minimum and increases to a maximum at 0.025 M, both the rate and the amount being greater." This apparently agrees with Fischer's work on adsorption of hydrochloric acid by gelatine, although the maximum concentration obtained here is slightly higher than that obtained by Fischer. This might well be due to the hydrogen-ion concentration of the gelatine employed. It will be remembered that the writer obtained greatest swelling with gelatine in N/20 hydrochloric acid, though lower concentrations were not used.

## II. EXPERIMENTS ON GAS ADSORPTION

While the general question of gas interchange has been a subject of interest for some time, and its connection with photosynthesis and metabolism has long been realized, the relation which the colloidal mass of the cell might bear to gases, purely as a matter of occlusion, has had little attention. The question is not one of gas adsorption by a simple chemical substance, for plant cells are composed of many complex materials, being normally acid in content, and the electrolytes that they contain modify this occlusion to some degree. Neither is the colloidal mass a purely passive medium, but it should be remembered that the reactions in living matter are very unstable, changing from minute to minute, so that stable equilibrium cannot be attained.

In considering gas adsorption by the cell it must be borne in mind that gases are not to be thought of as being adsorbed as such; they pass into aqueous solution and are then adsorbed, each cell being completely enveloped by

<sup>30</sup> Taylor, W. W., *Chemistry of colloids*. New York, 1915. P. 160–161.

a very thin water film, which is continuous with the water of imbibition in the colloid mass. Adsorption must therefore depend, to some degree, on the solubility of the gas in the liquid. For example, Weisner and Molisch<sup>31</sup> found that carbon dioxide diffused more rapidly than hydrogen or oxygen through vegetable membranes.

A number of the substances used for the present study of carbon-dioxide adsorption were of the same kinds as those employed for acid adsorption; namely, gels of agar (2 per cent), gelatine (10 per cent) acidified agar, and agar-stearate mixtures, cotyledons of Windsor bean and Lima bean, also pieces of potato tuber and carrot root. For agar, gelatine, acidified agar and the agar-stearate mixtures the procedure was as follows: 15 cc. of hot sol was poured into a weighed flask and after it had cooled and solidified the flask with its contents was reweighed. For carbon-dioxide adsorption the gas was run in by displacing water and the flask was corked, with a water seal, and placed in an inverted position for twenty-four hours, when the excess gas was displaced by alcohol (later water was used). The flask was then attached to the Pettenkofer apparatus and heated in boiling water for one hour. Most of the carbon dioxide came over in the first fifteen or twenty minutes. The amount of carbon dioxide in the substance was determined by difference in the barium hydroxide titration before exposing it to carbon dioxide and after doing so. When the material was acidified it was placed for twenty-four hours in acid solution before exposure to carbon dioxide.

The cotyledons were first soaked in water or acid and then suspended in such a manner that they did not touch one another. Fresh material of carrot and potato, except when acidified, was suspended immediately in a carbon-dioxide atmosphere. The potato tubers were cut in pieces about 2.5 cm. square and 1 mm. thick. The carrot roots were used both whole and cut into pieces of the same thickness as the pieces of potato tuber. The glass tubes used for exposing plant tissues to carbon dioxide were made of wide-bore (about 4.5 cm.) glass tubing, each tube having a ground glass stopper and wide lip for mercury seal at the upper end. The lower end of the tube was narrowed into a neck about 2 cm. in diameter. The ground glass stoppers bore glass hooks, from which was suspended the material, after having been soaked and strung on threads with glass beads between so that as much surface as possible would be exposed to the carbon dioxide. The gas was run in by displacing water, the lower end of the flask was corked with a water seal, and a mercury seal also employed at the top to insure against loss of carbon dioxide. The material was then left for twenty-four hours at a temperature of 22–24°C.

Controls not exposed to carbon dioxide were included in all tests, to determine whether the substance concerned gave off any carbon dioxide on heat-

---

<sup>31</sup> Wiesner, J., and H. Molisch, Untersuchungen über die Gasbewegung in der Pflanze. Sitzungsber. (Math.-Nat. Kl.) K. Akad. Wiss. Wien 98: 670–713. 1889.

ing, due either to decomposition at the high temperature used, or to possible carbon dioxide occluded in the case of the plant tissues. The gelatine controls, both the fresh gel as well as that soaked in water or hydrochloric acid

TABLE VII

*Average adsorption of water and excretion of acid, in grams per gram of substance, in 24 hours*

SUBSTANCE	WEIGHT USED	GAIN	WATER ABSORBED, PER GRAM	N/10 NaOH TO NEUTRALIZE 10 CC. OF SOLUTION AFTER ABSORPTION	ACID EXCRETED, IN TERMS OF N/10 HCl	ACID EXCRETED PER GRAM, IN TERMS OF N/10 HCl	NUMBER OF TESTS
	g.	g.	g.	cc.	cc.	cc.	
Gelatine.....	2.694	10.175	3.819	0.100	0.0033	0.0012	11
Agar-gelatine mixture.....	2.557	18.167	7.125	0.100	0.0030	0.0012	8
Phaseolus cotyledons.....	2.095	2.364	1.128	0.427	0.0151	0.0072	11
Vicia cotyledons.....	2.214	2.763	1.251	0.274	0.0097	0.0044	10
Lupinus cotyledons.....	2.064	3.075	1.485	0.266	0.0094	0.0046	7
Starch.....	1.667	1.524	0.916	—	—	—	8
Agar.....	1.384	0.780	7.770	—	—	—	2
Coconut endosperm.....	0.410	1.864	4.540	—	—	—	6

TABLE VIII

*Adsorption of carbon dioxide by glycocoll-agar, malic acid-agar, succinic acid-agar and nucleinic acid-agar, in 24 hours\**

	WEIGHT USED	CARBON DIOXIDE ADSORBED	CARBON DIOXIDE ADSORBED, PER GRAM OF GEL.
	g.	mg.	mg.
Glycocoll, 0.9%; agar, 2%—2 tests.....	14.32 14.84	17.75 27.00	1.24 1.82
Average of 2 tests.....			1.53
Glycocoll, 0.2%; agar, 2%.....	15.00	25.50	1.70
Nucleinic acid, 0.2%; agar, 2%.....	14.62	23.50	1.61
Gelatine, 20 parts; agar, 80 parts.....	13.61	27.50	2.02
Malic acid, 0.9%; agar, 2%—2 tests.....	14.45 14.04	25.00 28.00	1.73 1.99
Average of 2 tests.....			1.86
Malic acid, 0.1%; agar, 2%.....	15.14	24.50	1.62
Succinic acid, 0.2%; agar, 2%.....	14.87	23.00	1.55
Succinic acid, 0.9%; agar, 2%.....	13.97	24.10	1.73
Succinic acid, 9.0%; agar, 2%.....	14.34	30.00	2.09

\* Blanks of glycocoll-agar, malic acid-agar and succinic acid-agar evolved no carbon dioxide, or only a trace.

solution, gave off no carbon dioxide on heating, or so little that the amount was within the experimental error of the method. This is likewise true of pure agar gel (as well as that soaked in hydrochloric acid solution), glyco-

TABLE IX

*Average adsorption of carbon dioxide, per gram of substance, in 24 hours, from atmosphere containing 94-95 per cent of carbon dioxide<sup>a</sup>*

SUBSTANCE	WEIGHT USED	CARBON DIOXIDE ADSORBED	CARBON DIOXIDE ADSORBED PER GRAM		NUMBER OF DETERMINATIONS
			Uncorrected	Corrected	
	g.	mg.	mg.	mg.	
Agar (2.5%) in CO <sub>2</sub> .....	14.352	26.44	1.85	—	10
Agar (2.5%) in N/100 HCl and CO <sub>2</sub> .....	14.426	25.83	1.79	—	3
Agar (2.5%) in N/50 HCl and CO <sub>2</sub> .....	14.385	25.63	1.85	—	2
Agar (2.5%) in N/20 HCl and CO <sub>2</sub> .....	14.394	25.55	1.77	—	10
Agar (2.5%) in N/10 HCl and CO <sub>2</sub> .....	14.471	23.94	1.65	—	16
Agar (2.5%) in N/5 HCl and CO <sub>2</sub> .....	14.431	23.98	1.66	—	7
Agar (2.5%) in N/2 HCl and CO <sub>2</sub> .....	14.525	24.94	1.72	—	7
Gelatine (10%) in CO <sub>2</sub> .....	14.825	23.50	1.58	—	6
Gelatine (10%) in H <sub>2</sub> O and CO <sub>2</sub> .....	14.270	25.42	1.78	—	3
Gelatine (10%) in N/10 HCl and CO <sub>2</sub> .....	14.775	37.25	2.52	—	6
Vicia cotyledons in H <sub>2</sub> O.....	13.718	15.67	1.14	—	3
Vicia cotyledons in H <sub>2</sub> O and CO <sub>2</sub> .....	4.290	16.15	3.76	2.63	10
Vicia cotyledons in N/10 HCl.....	4.438	1.88	0.43	—	4
Vicia cotyledons in N/10 HCl and CO <sub>2</sub> .....	4.363	12.55	2.90	2.48	11
Phaseolus cotyledons in H <sub>2</sub> O.....	7.044	4.50	0.64	—	3
Phaseolus cotyledons in H <sub>2</sub> O and CO <sub>2</sub> .....	2.505	8.85	3.53	2.90	5
Phaseolus cotyledons in N/10 HCl.....	6.785	2.38	0.35	—	2
Phaseolus cotyledons in N/10 HCl and CO <sub>2</sub> .....	2.292	7.71	3.40	3.04	4
Potato tuber, pieces.....	15.653	3.33	0.21	—	3
Potato tuber in CO <sub>2</sub> .....	18.502	39.25	2.13	1.92	11
Potato tuber in N/10 HCl.....	13.966	2.38	0.17	—	2
Potato tuber in N/10 HCl and CO <sub>2</sub> .....	13.610	16.00	1.18	1.01	5
Carrot root, whole.....	30.202	8.33	0.28	—	3
Carrot root, whole, in CO <sub>2</sub> .....	20.502	42.32	2.19	1.91	6
Carrot root, pieces, in CO <sub>2</sub> .....	28.407	59.00	2.09	—	2
Agar (2%) with sodium stearate (0.5%) in CO <sub>2</sub> .....	14.402	30.25	2.10	—	4
Agar (2%) with sodium stearate (0.9%) in CO <sub>2</sub> .....	14.420	37.63	2.61	—	2
Agar (2%) with sodium stearate (1%) in CO <sub>2</sub> .....	15.513	41.00	2.65	—	4
Agar (2%) with sodium stearate (10%) in CO <sub>2</sub> .....	14.768	82.09	5.57	—	8
Sodium stearate (10%) in CO <sub>2</sub> .....	14.742	110.54	7.49	—	4

<sup>a</sup> Untreated agar and gelatine gels, as well as those soaked in acids, gave off no carbon dioxide, or but a trace.

agar and malic and succinic acid agar. Both kinds of bean cotyledons gave off carbon dioxide after standing twenty-four hours in water or N/10 hydrochloric acid. The amount given off in acid is less than that given off in

distilled water (table IX). The cause of this relation has not been investigated, but it appears that there are two possibilities: (1) that there are substances in the cotyledons which are broken down when heated, so that carbon dioxide is driven off, or (2) that the amount of carbon dioxide in the cotyledons may be greater in water than in acid, since carbon dioxide is more soluble in water than in solutions of hydrochloric acid. In the first case some carbon dioxide might be evolved before heating, and therefore its amount might appear lower. In the second case the acid might displace some of the carbon dioxide before the amount of the gas was determined. These two suggestions are advanced simply as possibilities. Of the two, the latter is probably the more likely, particularly in relation to Greffcken's<sup>32</sup> work, which shows that electrolytes depress the solubility of gases in water. The fact remains, however, that more carbon dioxide is evolved on heating cotyledons placed in water than on similarly heating those in acid. When a correction is made for the amount of carbon dioxide present in cotyledons before exposure to the gas, the actual amount of carbon dioxide adsorbed is found to be slightly more in water-soaked than in acid-soaked cotyledons, per gram of dry weight. The same is true of pieces of potato tuber, although when soaked in N/10 hydrochloric acid solution the pieces became plasmolysed. On account of the difficulty here suggested no acid determinations were made with carrot roots.

Hydrochloric acid has little or no effect on the amount of carbon dioxide adsorbed per gram of agar (table IX); there is perhaps a slight decrease with increase in acid concentration. With gelatine there is a difference in the amount of carbon dioxide adsorbed depending on whether the gelatine has been soaked or not. Table II shows the increase in weight of the gelatine strips after treatment in water or N/10 hydrochloric acid for twenty-four hours.

If the average amounts of carbon dioxide actually adsorbed by the gelatine strips from water and from N/10 hydrochloric acid are compared with the amounts that might have been held in solution by the water that entered the strips during the test period, it appears that carbon-dioxide adsorption was about proportional to water adsorption, as is shown by the following tabulation. The values are derived from those of tables II and IX.

	CARBON DIOXIDE OBTAINED PER GRAM	CARBON DIOXIDE, CALCULATED PER GRAM
	<i>ml.</i>	<i>mg.</i>
Fresh gelatine gel (not soaked in water).....	1.58	
Gelatine gel after 24 hours in water.....	1.78	1.76
Gelatine gel after 24 hours in N/10 hydrochloric acid.....	2.52	2.72

<sup>32</sup> Greffcken, Gustav, Beiträge zur Kenntnis der Löslichkeitsbeeinflussung. Zeitschr. Phys. Chem. 49: 257-302. 1904.

In the case of hydrochloric acid the calculated amount of carbon dioxide is seen to be somewhat higher than that actually adsorbed, which may be due to the fact that hydrochloric acid decreases the solubility of carbon dioxide in water. This is indicated by Greffcken's results, mentioned above.

Findlay and Creighton<sup>33</sup> found gelatine sols increased the solubility of carbon dioxide in water, whereas other emulsoids tested (except ferric oxide sol) decreased it; and Siegfried<sup>34</sup> found that amino acids formed compounds with carbon dioxide.

The organic acids employed in the present studies were some of those commonly found in plant tissues, malic acid being one of the acids largely formed in the cacti as a product of the incomplete oxidation due to their peculiar type of respiration. Increasing the concentration of malic or succinic acid apparently slightly increased the amount of carbon dioxide absorbed by agar (table VIII). From a few experiments with glycocoll the reverse seems to be indicated. Only one determination was made with nucleinic acid.

MacDougal<sup>35</sup> added "Ivory" soap to mixtures of agar and gelatine and employed these mixtures for some of his swelling experiments. He advanced the view that protoplasm might be essentially a complex pentosan—albumin-soap mixture, the soap forming films about the particles of the more solid phase, and concluded that soaps were very sensitive to hydrogen-ion concentration. For this reason it was thought that salts of fatty acids might exert marked influence on carbon-dioxide adsorption by a gel. The influence of sodium stearate upon the adsorption of carbon dioxide by agar gel was therefore tested in these studies. While salts of fatty acids are found in plants they do not occur there in the concentrations employed here for sodium stearate.

The amount of carbon dioxide adsorbed by agar with sodium stearate increases with the concentration of the stearate in the mixture. What the actual effect of the carbon dioxide is on the sodium stearate gel was not ascertained. If the stearate is hydrolyzed sufficiently in the gel chemical interaction between it and carbon dioxide may take place, since sodium stearate is a salt of a weak acid and a strong base, or the relation just mentioned may represent a case of simple adsorption.

According to the results, malic acid and succinic acid, in low concentrations, do not increase the quantity of carbon dioxide adsorbed above that absorbed by pure agar. If there is any inclination to variation in the amount of carbon dioxide adsorbed by agar soaked in hydrochloric acid solutions, it is rather a decreasing than an increasing adsorption with increase in acid con-

<sup>33</sup> Findlay, Alexander, and Henry Germain Maude Creighton, The influence of colloids and true suspensions on the solubility of gases in water. Part I, Solubility of carbon dioxide and nitrous oxide. Trans. Chem. Soc. 97: 536-561. 1910.

<sup>34</sup> Siegfried, M. Ueber die Bindung von Kohlensäure durch Amphotere Amidokörper. Zeitschr. physiol. Chem. 44: 85-96. 1905.

<sup>35</sup> Loc. cit., see note 27.

tent. Glycocoll appears to have no particular effect in the concentrations used. It is to be noted that the concentrations of organic acids employed were low, except the 9 per cent succinic acid, and that the determinations with these organic compounds were few. Further work is therefore needed.

Neither hydrochloric acid nor the organic acids tested, in the concentrations employed, showed any considerable influence on the adsorption of carbon dioxide by agar. Gelatine showed an increased carbon-dioxide content when acidified, but this appears to be referable to its water capacity, which is increased greatly by hydrochloric acid. Sodium stearate evidently increases the carbon dioxide apparently adsorbed by agar. Tenth normal hydrochloric acid decreases the amount of carbon dioxide taken up by the cotyledons tested and by potato tissue. This is probably due to the fact that carbon dioxide is less soluble in hydrochloric acid solution than in water.

It will be noticed that the acid concentrations employed, particularly those in the acid adsorption experiments in the first part of this paper, are higher than those found in most plant tissues, but it should be remembered that the acid concentration of the cacti, particularly at certain times of day, is unusually high. Richards<sup>36</sup> found that the average acidity (in cc. of N 10 sodium hydroxide per gram of fresh material) in July, in year-old tissue of *Opuntia versicolor* varied from 0.74 in the morning to 0.29 in the afternoon, or from 3.75 to 1.57 cc. per gram of dry material. The acidity is also higher in young than in mature tissue. From some other determinations on acidity in the same work, the total acidity for *Opuntia versicolor* taken in April averaged from 1.48 cc. to 0.48 cc. of N/10 sodium hydroxide per gram of fresh material, depending on the time of day. The acidity of expressed juice in one case (in the morning) was equal to 1.98 cc. of N 10 sodium hydroxide. This is almost equivalent to 1 cc. of N 5 hydrochloric acid solution.

From some figures taken from manuscript kindly loaned by Dr. Richards, the acidity of non-succulents such as *Richardia* (calla-lily) petioles and leaves varied from 0.2 cc. to 0.3 cc. of N/10 sodium hydroxide per gram of fresh material. The corresponding value for *Ipomaea* (morning-glory) was 0.2 cc. These figures agree very closely with values taken at random from Warburg's paper<sup>37</sup> and reduced to the same terms. It will be seen that they are much lower than those for similar acid determinations in the cacti.

As to the cause or variation in acid content between day and night, Warburg considered this as related to the fact that plants such as succulents, which are so well protected against loss of water by transpiration are also limited in their gas exchange, with consequent increase in acid production due to

<sup>36</sup> Richards, Herbert M., Acidity and gas interchange in the cacti. Carnegie Inst. Washington Publ. 209 Washington, 1915.

<sup>37</sup> Warburg, O., Ueber die Bedeutung der organischen Säuren für den Lebensprozess der Pflanzen, speciell der Sog. Fettpflanzen. Untersuch. Bot. Ints. Tübingen 2: 53-150. 1886.

incomplete oxidation. Purjewicz<sup>38</sup> looks upon the splitting of acids as an oxidation process, whereas Kraus<sup>39</sup> considered it a by-product of respiration. Both Warburg and Pfeffer<sup>40</sup> regarded the breaking up of acids as a source of carbon dioxide for photosynthesis. Aubert<sup>41</sup> corroborated the work of earlier investigators, and made a more exhaustive study of the respiration of succulents.

Richards<sup>42</sup> concluded that formation of malic acid in the cacti takes place at all times, due to partial oxidation, but that during the day this acid is broken down, owing to the light and higher temperature, and so it does not accumulate in the tissues. He likewise concluded that light is a more important factor than temperature in decreasing acidity. In a later paper by MacDougal, Richard and Spoehr<sup>43</sup> succulence is considered as a characteristic of plants that form pentoses easily from polysaccharides.

It is to be remembered that variation in the  $\text{CO}_2\text{-O}_2$  ratio and the formation of acid in cacti go hand in hand. With rising acidity the carbon-dioxide production is relatively low and the oxygen absorption is high, so that the ratio of carbon dioxide to oxygen is less than unity; with light and rising temperature carbon-dioxide evolution is accelerated and the ratio value becomes nearer unity. At night, when the temperature is low, the acid content of cactus tissues is high, and little carbon dioxide is given off. It is at this time that the condition in the tissue might be said to simulate, very roughly, that of the acidified gels of the present studies. With light and rising temperature the acidity decreases and there is greater evolution of carbon dioxide. This might be expected from the photolytic action of light on the acid formed during the night. Also, increase in temperature of course decreases the solubility of the gas. It is obvious, therefore, that there are many causes other than acidity influencing carbon dioxide emission.

The conclusion arrived at from the results of the experiments dealt with in this paper is that acidity decreases rather than increases the amount of carbon dioxide adsorbed, at least with the acids and in the concentrations here employed. Although it might have been predicted that acid would lessen the gas adsorption of substances, since it is known that gases are less soluble in acid solutions than in pure water, this has not been experimentally previously demonstrated for the type of substances employed here. While this work does not deal with gas interchange itself, the fact that acidity does not greatly influence but, if it is influential at all, tends to lessen the

<sup>38</sup> Purjewicz, K., Die Bildung und Zersetzung der organischen Säuren bei den höheren. Pflanzen. Bot. Centralbl. 58: 368-374. 1894.

<sup>39</sup> Kraus, Gregor. Über die Wasservertheilung in der Pflanze. IV, Die Acidität des Zellsaftes. Abh. Naturforsch. Ges. Halle. 16: 143-205. 1884.

<sup>40</sup> Pfeffer, W., Physiology of plants. Translated by A. J. Ewart. Oxford 1: 326, 1900. See vol. 1, p. 326.

<sup>41</sup> Aubert, M., Recherches physiologiques sur les plantes grasses. (Dissertation, Université de Paris.) Paris, 1892.

<sup>42</sup> *Loc. cit.*, see note 36.

<sup>43</sup> MacDougal, D. T., H. M. Richards and H. A. Spoehr, Bas is of succulence in plants. Bot. Gaz. 57: 405-415 1919.

carbon dioxide adsorption (at least for the concentrations used here) makes it appear unnecessary to consider it as contributory to the peculiar  $\text{CO}_2\text{-O}_2$  ratio of succulent plants. It seems that the causes for this peculiar ratio value must be looked for elsewhere.

This work was done in the Botanical Laboratory, Barnard College, and the writer is indebted to Dr. Herbert M. Richards for his advice and interest, which are greatly appreciated.

BOTANICAL LABORATORY

BARNARD COLLEGE

COLUMBIA UNIVERSITY

### LITERATURE CITED

- AUBERT, M., *Recherches physiologiques sur les plantes grasses*. (Dissertation, Université de Paris.) Paris, 1892.
- BLYTH, ALEX., AND MERIDITH W. BLYTH, *Foods, their composition and analysis*. New York, 1903.
- BOROWIKOW, G. A., Ueber die Ursachen des Wachstums der Pflanzen. *Zeitschr. Kolloide* 15: 27-30. 1914.
- CAREY, CORNELIA L., A method of preparation and some properties of a starch gel. *Bull. Torrey Bot. Club* 47: 455-463. 1920.
- DACHNOWSKI, A., The effects of acid and alkaline solutions upon the water relation and the metabolism of plants. *Amer. Jour. Bot.* 1: 412-439. 1914.
- DONNAN, F. G., Theorie der Membrangleichgewichte und Membranpotentiale bei vorhandenem von nichtdialysierenden Elektrolyte. *Zeitschr. Elektro-Chem.* 17: 572-581. 1911.
- DONNAN, F. G., AND A. B. HARRIS, The osmotic pressure and conductivity of aqueous solutions of congo red, and reversible membrane equilibria. *Trans. Chem. Soc.* 99: 1554-1577. 1911.
- FINDLAY, ALEXANDER, AND H. G. M. CREIGHTON, The influence of colloids and fine suspensions on the solubility of gases in water. Part I, Solubility of carbon dioxide and nitrous oxide. *Trans. Chem. Soc.* 97: 536-561. 1910.
- FISCHER, MARTIN H., *Oedema and nephritis*. New York, 1915.
- GREFFCKEN, GUSTAV, Beiträge zur Kenntnis der Löslichkeitsbeeinflussung. *Zeitschr. Phys. Chem.* 49: 257-302. 1904.
- HEYNE, B., On the deoxidation of the leaves of *Cotyledon calycina*, in a letter to A. B. Lambert, Esq. *Trans. Linn. Soc.* 11<sup>2</sup>: 213-215. 1815.
- KRAUS, G., Ueber die Wasservertheilung in der Pflanze. IV, Die Acidität des Zellsaftes. *Abh. Naturforsch. Ges. Halle* 16: 143-205. 1884.
- KÖNIG, K., *Chemie der menschlichen Nahrungs und Genussmittel*. Vol. I, p. 403. Berlin, 1893.
- LEIGHTON, ALAN, Adsorption of acids by cellulose. *Jour. Phys. Chem.* 20: 188-194. 1916.
- LINK, H. F., Zusatz von H. F. Link, ueber die Desoxydation der Blätter von *Cotyledon calycina* B. Heyne, in einem Briefe von A. B. Lambert. *Jahrb. für Gewächskunde* 1<sup>2</sup>: 73-76. 1820.
- LYDD, DOROTHY JORDAN, On the swelling of gelatin in hydrochloric acid and caustic soda. *Biochem. Jour.* 14: 147-170. 1920.
- LYDD, HOYES, Adsorption of some substances by starches. *Jour. Amer. Chem. Soc.* 33: 1213-1226. 1911.
- LOEB, JACQUES, Proteins and colloid chemistry. *Science* 52: 449-456. 1920.
- LONG, ESMOND R., Growth and colloid hydration in cacti. *Bot. Gaz.* 59: 491-497. 1915.
- MACDOUGAL, D. T., Imbibitional swelling of plants and colloidal mixtures. *Science* 44: 502-505. 1916.
- The effects of acids and salts on "biocolloids." *Science* 46: 269-272. 1917.
- The effects of age and the inclusion of salts on the heterotrophic action of colloidal bodies of cytological interest. *Proc. Soc. Biol. and Med.* 18: 244-246. 1921.
- Water deficit and action of vitamins, amino compounds and salts on hydration. *Amer. Jour. Bot.* 8: 296-302. 1921.
- MACDOUGAL, D. T., H. M. RICHARDS AND G. A. SPOEHR, Basis of succulence in plants. *Bot. Gaz.* 57: 405-416. 1919.
- OSTWALD, WOLFGANG, Ueber den Einfluss von Säuren und Alkalien auf die Quellung von Gelatine. *Arch. Physiol.* 108: 563-589. 1905.
- PFEFFER, W., *Physiology of plants*. Translated by A. J. Ewart. Oxford, 1900.
- PROCTER, HENRY R., Über die Einwirkung verdünnter Säuren und Salzlösungen auf Gelatine. *Kolloidechem. Beih.* 2: 243-284. 1911.

- PURJEWICZ, K., Die Bildung und Zersetzung der organischen Säuren bei den höheren Pflanzen. Bot. Centralbl. 58: 368-374. 1894.
- RICHARDS, HERBERT M., Acidity and gas interchange in the caeti. Carnegie Inst. Washington Publ. 209, Washington, 1915.
- SCHULZE, E., E. STEIGER AND W. MAXWELL, Untersuchung über die chemische Zusammensetzung einiger Leguminosensamen. Landw. Versuchsstat. 39: 269-326. 1891.
- SIEGFRIED, M., Über die Bindung von Kohlensäure durch amphotere Amidokörper. Zeitschr. physiol. Chem. 44: 85-96. 1905.
- SHULL, CHARLES A., Temperature and the rate of moisture intake in seeds. Bot. Gaz. 69: 361-390. 1920.
- SHREVE, EDITH B., Investigations on the imbibition of water by gelatine. Science 48: 324-327. 1918.
- TAYLOR, W. W., Chemistry of colloids. New York, 1915.
- THOMAS, ARTHUR W., Tabulation of the hydrogen and hydroxyl ion concentrations of some acids and bases. Jour. Amer. Leather Chemists Assoc. 15: 133-146. 1920.
- WARBURG, O., Ueber die Bedeutung der organischen Säuren für den Lebensprozess der Pflanzen, speciell der sog. Fettpflanzen. Untersuch. Bot. Inst. Tübingen 2: 53-150. 1886.
- WIESNER, J., AND H. MOLISCH, Untersuchungen über die Gasbewegung in der Pflanze. Sitzungsber. (Math.-Nat. Kl.) K. Akad. Wiss. Wien 98: 670-713. 1889.

# PHYSIOLOGICAL RESEARCHES

## VOLUME 1

- No. 1.* Caldwell, Joseph Stuart, The relation of environmental conditions to the phenomenon of permanent wilting in plants. Fourteen figures, showing graphs. *Physiol. Res.* **1**: 1-56. July, 1913. Price \$.75.
- No. 2.* Hawkins, Lon A., The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. Six figures in text. *Physiol. Res.* **1**: 57-91. August, 1913. Price \$.50.
- No. 3.* Wuist, Elizabeth Dorothy, Sex and development of the gametophyte of *Onoclea struthiopteris*. Fifteen figures. *Physiol. Res.* **1**: 93-132. September, 1913. Price \$.60.
- No. 4.* Totttingham, William E., A quantitative chemical and physiological study of nutrient solutions for plant cultures. Fifteen figures. *Physiol. Res.* **1**: 133-245. May, 1914. Price \$1.10.
- No. 5.* Lehenbauer, Philip Augustus, Growth of maize seedlings in relation to temperature. Four figures, showing graphs. *Physiol. Res.* **1**: 247-238. December, 1914. Price \$.60.
- No. 6.* MacDougal, D. T., E. R. Long and J. G. Brown, End results of desiccation and respiration in succulent plants. Five figures in text and three plates. *Physiol. Res.* **1**: 289-325. August, 1915. Price \$.80.
- No. 7.* Shive, J. W., A study of physiological balance in nutrient media. Fifteen figures. *Physiol. Res.* **1**: 327-397. February, 1916. Price \$.90.
- No. 8.* Livingston, Burton Edward, Physiological temperature indices for the study of plant growth in relation to climatic conditions. Four figures, one showing graphs, three showing chart. *Physiol. Res.* **1**: 399-420. April, 1916. Price \$.40.
- No. 9.* Livingston, Burton Edward, A single index to represent both moisture and temperature conditions as related to plant growth. One figure, showing chart. *Physiol. Res.*, **1**: 421-440. May, 1916. Price \$.40.
- No. 10.* Hibbard, R. P., and O. E. Harrington, Depression of the freezing-point in triturated plant tissues, and the magnitude of this depression as related to soil moisture. *Physiol. Res.* **1**: 441-454. August, 1916. Price \$.38.

VOLUME 1, COMPLETE, PRICE \$5.00

# PHYSIOLOGICAL RESEARCHES

## VOLUME 2

- No. 11.* Harris, J. Arthur, and John V. Lawrence, with the coöperation of Ross Aiken Gortner, The cryoscopic constants of expressed vegetable saps, as related to environmental conditions in the Arizona deserts. *Physiol. Res.* 2: 1-49. July, 1916. Price \$1.00.
- No. 12.* Bakke, A. L., and B. E. Livingston, Further studies of foliar transpiring power in plants. Three figures, two showing graphs. *Physiol. Res.* 2: 51-71. August, 1916. Price \$.45.
- No. 13.* Shreve, Edith B., An analysis of the causes of variations in the transpiring power of cacti. Ten figures, showing graphs. *Physiol. Res.* 2: 73-127. September, 1916. Price \$1.10.
- No. 14.* McLean, Forman T., A preliminary study of climatic conditions in Maryland, as related to plant growth. (Carried out under the auspices of the Maryland State Weather Service, in 1914.) Fourteen figures, ten showing graphs. *Physiol. Res.* 2: 129-208. February, 1917. Price \$1.60.
- No. 15.* Folsom, Donald, The influence of certain environmental conditions, especially water supply, on form and structure in *Ranunculus*. Twenty-four figures, mostly diagrams and graphs. *Physiol. Res.* 2: 209-276. December, 1918. Price \$1.70.
- No. 16.* Long, Frances Louise, The quantitative determination of photosynthetic activity in plants. *Physiol. Res.* 2: 277-300. August, 1919. Price \$.75.
- No. 17.* Dunn, Grace A., A comparative study of the two races of *Rhizopus nigricans*. One figure, photograph. *Physiol. Res.* 2: 301-339. April, 1921. Price \$1.50.
- No. 18.* Hildebrandt, F. Merrill, A study of the climatic conditions of Maryland, as measured by plant growth. Eight figures, seven showing graphs and five of these in two colors. *Physiol. Res.* 2: 341-405. May, 1921. Price \$2.75.
- No. 19.* Carey, Cornelia L., The relation of acidity to carbon-dioxide adsorption by certain gels and plant tissues. *Physiol. Res.* 2: 407-432 May, 1923. Price \$1.25









169708

Author    Physiological Researches. 1916-23. Vol. 2.

Title .....

**University of Toronto  
Library**

**DO NOT  
REMOVE  
THE  
CARD  
FROM  
THIS  
POCKET**

Acme Library Card Pocket  
Under Pat. "Ref. Index File"  
Made by LIBRARY BUREAU

